LINKAGE GROUPS OF THE JAPANESE MORNING GLORY*

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Received July 11, 1928

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INTRODUCTION

Nearly one hundred genes have been detected in the Japanese morning glory, *Pharbitis Nil*. Their inter-relations, however, are not yet fully determined. In this paper the writer analyzes the arrangement of 34 genes in ten linkage groups. Crossing over takes place in macro-sporogenesis and in microsporogenesis, with a frequency that is apparently the same for both. At present, owing to the fact that the calculations are based on data obtained by self-fertilization, the writer does not expect to give a final decision on the relative frequencies of crossing over in the two types of sporogenesis nor to give precise values for the crossing over intervals. The estimations of the recombination frequencies are made according to the formula proposed by TAKEZAKI (1925). For some genes the symbols that were adopted in the writer's previous papers have now been changed to bring them under a single system of designation.

The writer takes pleasure in expressing hearty thanks to Professor K. MIYAKE for his generosity in providing every facility for the present investigation, and to Mr. K. HASHIMOTO for his financial aid. The author's

* Part of the cost of the accompanying tables is paid for by the GALTON AND MENDEL MEMORIAL FUND.

acknowledgments are also due to Professor T. H. MORGAN, Doctor C. B. BRIDGES, Doctor A. H. STURTEVANT and Doctor ALEXANDER WEINSTEIN for aid given in the preparation of this manuscript.

VARIEGATED LINKAGE GROUP

The first linkage case found in the Japanese morning glory was that of variegated and crumpled (formerly termed punched) leaves (HAGIWARA 1919; IMAI 1919), this being confirmed by later work (IMAI 1921b, 1924, 1925, 1926; HAGIWARA 1921, 1922a). Two different crumpled leaf characters were found (IMAI 1926). One of these, crumpled–1 (c_1, formerly u^v), is linked to variegation. Table 1 contains the complete F_2 data on the

TABLE 1

F_2 from the cross of variegated crumpled-1 ($v\ c_1$) by normal.

CROSS	+	v	c_1	$v\ c_1$	TOTAL
RA×21–2	50	9	12	8	79
RA×M1	70	9	10	11	100
β×318	133	15	15	41	204
A×71–2	83	10	19	27	139
β×73	130	11	10	20	171
SG×RA	46	10	7	12	75
M15×50	938	120	118	232	1408
Total	1450	184	191	351	2176

Certain F_2 families showing marked inviability effects are omitted from this table.

TABLE 2

F_2 from cross 324×316, involving variegated and crumpled-1.

F_3 PARENT	GENOTYPE	NO. FAMILIES	+	v	c_1	$v\ c_1$	TOTAL
Normal	+ + / + +	16	482	..	..	..	482
	v + / + +	4	125	39	..	..	164
	+ c_1 / + +	6	145	..	48	..	193
	$v\ c_1$ / + +	26	515	46	48	139	748
	v + / + c_1	3	58	24	22	0	104
Variegated	$v\ c_1$ / v +	1	..	14	..	5	19
Crumpled-1	$v\ c_1$ / + c_1	5	..	..	109	40	149
Variegated crumpled-1	$v\ c_1$ / $v\ c_1$	8	..	..	..	70	70

coupling combination for the two genes *v* (variegated) and c_1 (crumpled –1), as obtained by the writer to date.

TABLE 3

F_2 from the cross of variegated and crumpled-1.

CROSS	+	*v*	c_1	*v* c_1	TOTAL
319×170	98	42	29	1	170
α×65	64	24	17	3	108
170×A2	162	67	82	6	317
170×77–1	36	13	14	0	63
170×65	155	65	69	7	296
M2×58–2	66	30	33	0	129
M2×M4	67	33	38	4	142
314×A2	163	75	45	2	285
S3×S5	33	13	17	0	63
65×505	211	91	82	2	386
189×921	224	88	89	1	402
187×921	86	43	45	1	175
Total	1365	584	560	27	2536

The amount of recombination due to crossing over between *v* and c_1 is 19.2 percent. Further examination of the coupling combination was made in F_2 with cross 324×316[1], which is shown in table 2.

TABLE 4

F_2 from cross 65×505, involving variegated and crumpled-1.

F_1 PARENT	GENOTYPE	NO. FAMILIES	+	*v*	c_1	*v* c_1	TOTAL
Normal	+ + / + +	1	74	..	..	..	74
	v + / + +	8	518	169	..	..	687
	+ c_1 / + +	2	88	..	21	..	109
	v + / + c_1	19	936	375	367	19	1697
	v c_1 / + +	3	196	22	18	38	274
Variegated	*v* + / *v* +	10	..	602	..	..	602
	v c_1 / *v* +	4	..	127	..	51	178
Crumpled-1	+ c_1 / + c_1	6	..	..	719	..	719
	v c_1 / + c_1	5	..	..	115	33	148

Of the 29 F_3 families in table 2 that gave dihybrid segregation, 26 repeated the previous combination, that is, coupling; whereas the other 3

[1] The F_2 linkage data from cross 324×316 are omitted from table 1 on account of marked deviation in viability.

changed into repulsion. In table 3 further F_2 data obtained from repulsion combinations are given.

The observed frequencies of the four forms, as given in table 3, show the recombination value to be 25.0 percent, which is somewhat larger than the figure from the coupling F_2 data. From one of these F_2, cross 65×505, F_3 was examined, the data obtained being shown in table 4.

Of the 22 dihybrid F_2 families of table 4, 19 showed repulsion combination and the rest showed coupling.

From the above tables the total available data for coupling are:

SOURCE	+	v	c_1	vc_1	TOTAL
Table 1	1450	191	184	351	2176
Table 2	515	46	48	139	748
Table 4	196	22	18	38	274
Total	2161	259	250	528	3198

The frequency of recombination, calculated on the basis of these figures, is 17.7 percent. The available data for repulsion give:

SOURCE	+	v	c_1	vc_1	TOTAL
Table 2	58	24	22	0	104
Table 3	1365	584	560	27	2536
Table 4	936	375	367	19	1697
Total	2359	983	949	46	4337

The recombination percent is 25.7, which is greater than the 17.7 percent obtained from the data of the coupling series. The figures, as calculated from the repulsion data, have much less importance in determining the true value, because the result may be greatly influenced by the smallness of the class of the double recessive. From the foregoing it seems better to disregard the repulsion data and to conclude that there is about 17.7 percent recombination due to crossing over between v and c_1.

Blown leaf is the only dominant leaf form character found in this plant. Two different dominant blown characters are known, blown–1 and blown-2 (IMAI, in press). One of these, blown–1, which is the commonest form, results in a strongly blown leaf. SÔ and NISHIMURA (1919) first pointed out the existence of linkage between blown and variegated. In F_2 of the cross of variegated blown–1 by normal the writer obtained the repulsion data collected in table 5.

The totals in table 5 give 29.3 percent of recombination due to crossing over between the genes v and B_1.

The writer (1924) has already reported the data for linkage between crumpled–1 and blown–1, but he does not cite them here, because the classification was not always exact for the crumpled character. The existence

of linkage of c_1 and B_1 is quite evident. The three genes, v, c_1 and B_1, occupy loci in the same chromosome; their arrangement in linear order, however, is not yet clearly determined.

One of the fasciated genes, fasciated–3 (f_3, formerly f^3), is linked with v, with about 20–25 percent recombination, according to the writer's analysis (IMAI 1927c). Thus the variegated linkage group contains four genes, that is, v, c_1, B_1 and f_3.

TABLE 5

F_2 from the cross of variegated blown-1 (v B_1) by normal.

CROSS	B_1	v B_1	+	v	TOTAL
RA×M1	60	18	20	2	100
RA×IH	63	16	27	0	106
RA×17–2	137	63	82	7	289
RA×50	125	68	60	5	258
SG×RA	37	16	16	6	75
Total	422	181	205	20	828

HAGIWARA (1922a) considered that cordate falls in the variegated linkage group, but this does not seem to be the case. It belongs to a different group as the writer has already shown (IMAI 1924).

CORDATE LINKAGE GROUP

Sô and NISHIMURA (1919) analyzed the linkage between cordate leaf and feathered flower ("Shishi"). Their data were from repulsion experiments, and because of strong linkage they obtained no double recessives.

TABLE 6

F_2 from the cross of cordate (c_o) by semi-contracted (s_c).

CROSS	+	c_o	s_c	c_o s_c	TOTAL
318×YJ	221	99	115	3	438
A1×YJ	170	92	88	4	354
A1×A2	200	85	60	2	347
A4×A2	103	43	44	1	191
Total	694	319	307	10	1330

The cordate leaf (c_o, formerly h) is a simple recessive to the normal, three-lobed condition. The hybrids have leaves with roundish lobes, by which characteristic they can be easily distinguished from the homozygous

normal type. The feathered flower (f_e, formerly s_t) invariably accompanies an uneven or rolled leaf, and notwithstanding the fact that the feathered corolla acts as a practically complete recessive to the singleness of flower, we can identify the heterozygous plants by their slightly rolled leaves. Utilizing such heterozygotic manifestations for cordate and feathered, MIYAKE and IMAI (1921) studied the genetic relation between these two genes and found 1.2 percent of recombination for them.

Contracted ("Uzu") is one of the popular characters found in the Japanese morning glory; it affects the whole body of the plant, contracting every organ. Semi-contracted, in which the contraction is partial, is a less-known character, probably because of its inconspicuous expression. Semi-contracted (s_c, formerly d^h) is due to a recessive gene which is not allelomorphic to contracted; therefore, when they are crossed, we get F_1 that is normal. In F_2 the segregation gives 9 normal: 3 semi-contracted: 4 contracted (IMAI 1924).

TABLE 7

F_2 from the cross of feathered semi-contracted (f_e s_c) by normal.

CROSS	+	f_e	s_c	f_e s_c	TOTAL
500×YJ	457	43	42	83	625
A1×YJ	235	27	29	63	354
Total	692	70	71	146	979

Hybridizations made between cordate and semi-contracted produced normal F_1 hybrids. On selfing the F_1 flowers, the repulsion F_2 data of table 6 were obtained.

On the basis of the data of table 6 the frequency of recombination for cordate and semi-contracted is 18.6 percent.

As stated above, cordate and feathered are linked, giving 1. 2 percent of recombination, and cordate is also linked with semi-contracted, giving 18.6 percent of recombination. Therefore these three genes are located in the same chromosome. An important question is, what is the intensity of linkage for feathered and semi-contracted? The writer crossed feathered semi-contracted with normal and got normal F_1 hybrids. On selfing F_1 normals the coupling data shown in table 7 were obtained.

Calculation of the recombination frequency on the basis of the data of table 7, gives 17.1 percent for feathered and semi-contracted. This linkage demonstrates that the three genes, c_o, f_e and s_c, are in the same chromosome. With the amount of recombination known for c_o and f_e (1. 2), c_o and s_c

(18.6) and for f_e and s_c (17.1) it is most probable that the order of loci is c_o—f_e—s_c, since the c_o s_c recombination (18.6) is very nearly equal to the sum of the c_o f_e and f_e s_c recombination values (1.2+17.1=18.3) rather than being equal to the difference, which would have indicated an order f_e—c_o—s_c. The map-distance f_e s_c may be slightly greater than 17.1, since this distance is probably long enough to allow some double crossing over. Map-distance is greater than recombination percentage by an amount equal to twice the number of cases of double, or "even", crossing over that occur between the loci considered. The amount of this difference cannot be determined at present for the interval f_e—s_c, since no locus between them is known.

The writer (IMAI 1924) obtained data indicating that there is weak linkage for cordate leaf and crêpe leaf (formerly named crapy). The evidence, however, is not enough to be convincing.

YELLOW LINKAGE GROUP

In the F_2 progeny from a cross of yellow leaf with white flower by brownish flower, MIYAZAWA (1918) obtained unusual results, which were interpreted (IMAI 1921b) as due to repulsion of high degree between yellow leaf (y, formerly g) and dusky (d_y). Later, the present writer carried out experiments of both the coupling and the repulsion types and determined the frequency of recombination to be 1.0 percent (IMAI 1925).

ACUMINATE LINKAGE GROUP

Several genes which are concerned with the production or inhibition of the white margin on the corolla have been detected, among which margined–1 (M_{r1}, formerly F^a) and margined–2 (M_{r2}, formerly F^b) are complementary; that is, white margin is a double dominant type, and each single dominant gene produces no discernible effect by itself. Margined–1 is linked very closely with contracted (see next section). Margined–2 has close linkage relation with acuminate (nandina) leaf (a_c, formerly n_n). The frequency of recombination for a_c and M_{r2} is about 0.5 percent (IMAI 1927a).

A cross was made between a plant with normal leaves and normal blue flowers (No. 26) and a plant with acuminate leaves and red flowers (No. UN). The F_1 plants had normal leaves and flowers. In F_2 the red color was found to be a double recessive, purple magenta. There was linkage between the genes for acuminate and magenta, the F_2 data obtained being shown in table 8.

In table 8 the blue (+) and purple (p_r) flowered plants have been combined, and also the magenta (m_g) and purple magenta ($p_r m_g$) plants. The calculation based on the F_2 data of table 8 shows 21.7 percent of recombination for the genes a_c and m_g. F_3 families were raised from 157 F_2 plants, of which the progenies of 73 were grown in the field until their maturation, whereas the others, 84 in number, were recorded when the seedlings

TABLE 8

F_3 from cross 26×UN, showing linkage of acuminate (a_c) and magenta (m_g).

	+	a_c	m_g	$a_c m_g$	TOTAL
Observed	305	35	40	55	435

began to develop their first leaf between the cotyledons. We can easily identify the acuminate seedlings by the broadened connection of the cotyledons with their stalks. The purplish (+ and purple) and reddish (magenta and purple magenta) flowers have hypocotyls of their respective

TABLE 9

F_3 from cross 26×UN, showing linkage of acuminate and magenta.

F_2 PARENT	GENOTYPE	NO. FAMILIES	+	a_c	m_g	$a_c m_g$	TOTAL
Normal	+ + / + +	32	2052	..	..	..	2052
	a_c + / + +	12	622	168	..	..	790
	+ m_g / + +	17	1026	..	321	..	1347
	$a_c m_g$ / + +	55	2689	346	375	576	3986
	a_c + / + m_g	3	51	21	20	0	98
Acuminate	a_c + / a_c + ?	1	..	4	..	..	4
	$a_c m_g$ / a_c +	16	..	88	..	25	113
Magenta	+ m_g / + m_g	3	..	..	224	..	224
	$a_c m_g$ / + m_g	5	..	..	952	318	1270
Acuminate magenta	$a_c m_g$ / $a_c m_g$	13	..	..	..	188	188

colors, by which they can readily be distinguished from each other. Sometimes, however, the distinction is not clear. In such cases the classification is definitely determined by the help of the color of the cotyledon stalks. In table 9 the data of the offspring of these 157 F_2 plants are summarized.

Among 119 not-acuminate purplish F_2 plants, 58 families exhibited dihybrid segregation of the characters in question. Of these, 55 showed coupling, whereas the others, 3 in number, gave repulsion. Based on the coupling data of table 9 the frequency of recombination is 21.1 percent. If we calculate the linkage value from the available data through two generations (tables 8 and 9) the result is 21.2 percent. The three not-acuminate purplish families gave no double recessives in a total of 98; this is to be expected if there is close linkage.

From the fact that a_c and M_{r2} are linked with about 0.5 percent recombination, and a_c and m_g are linked with about 21.2 percent recombination we can regard these three genes (a_c, M_{r2} and m_g) as located in the same chromosomal set. Whether the M_{r2} locus is situated between the loci of a_c and m_g (order, a_c—M_{r2}—m_g) or lies to the left of acuminate (order, M_{r2}—a_c—m_g) has not yet been determined.

HAGIWARA (1923) concluded that four genes of which two, p (the writer's m_g) and b (the writer's p_r), are concerned with the production of flower color and the other two, t_1 and t_2, determine the distribution of the color inside of the flower tube, must all be located in this chromosome. But the classification of the flower tube patterns involved in HAGIWARA'S data is difficult, for which reason the writer prefers at the present time not to include HAGIWARA'S genes definitely in the acuminate group.

CONTRACTED LINKAGE GROUP

SÔ and NISHIMURA (1919) pointed out the existence of close linkage between contracted and white-margined flower. Later the writer (IMAI 1919, 1921b, 1925, 1927a) determined the frequency of recombination for the two genes contracted (c_t, formerly d) and margined-1 (M_{r1}), to be about 1.1 percent. On the basis of all available data (table 4, 1925) TAKEZAKI'S formula gives 1.0 percent recombination. HAGIWARA (1922b) obtained a similar result.

When a "parental stock"[1], No. 375–DA, was selfed, the S_1 (the first generation showing segregation) offspring exhibited segregation for three pairs of characters: white-margined flower (M_{r1}) *versus* the normal self-colored flower; intense flower color (i) *versus* the normal flower color; and stem colorless except for axils, that is interaxil-green (i_g) *versus* the normal colored stem. Usually strains with green stems have colorless axils and bear white flowers with either white tubes or colored ones. The interaxil-green is a rare strain that has colored axils and bears colored flowers.

[1] The term "parental stock" refers to a strain widely used by fanciers, and heterozygous for many character pairs.

Two generations of this "parental stock" show dependent assortment of three gene pairs in question. In table 10 we consider the data according to the segregation frequencies for interaxil-green and margined–1 alone.

In table 10 coupling linkage of interaxil-green and margined–1 occurred with a frequency of recombination of 6.6 percent. The S_2 (the second generation showing segregation) data, arranged with respect to interaxil-green and margined–1, is given in table 11.

TABLE 10

S_1 from plant 375–DA, showing coupling of interaxil-green (i_g) and margined-1 (M_{r1}).

	M_{r1}	$i_g M_{r1}$	+	i_g	TOTAL
Observed	65	2	3	17	87

Of 36 margined S_1 plants 13 bred true to this type, and the others, with one exception, gave rise to four types in coupling distribution. From the total data of 22 families, whose mother plants had a dihybrid constitution of $i_g+/+M_{r1}$, the frequency of recombination is 6.5 percent. On the basis of all the available data in these two generations the value is 6.5 percent. HAGIWARA (1926b), studying the same gene relation, recorded 53 margined, 6 interaxil-green margined, 5 type and 32 interaxil-green among 96 F_2 plants obtained from two crosses made between interaxil-green and margined. By TAKEZAKI'S formula these figures give 9.7 percent

TABLE 11

S_2 from plant 375-DA, showing linkage of interaxil-green and margined-1.

S_1 PARENT	GENOTYPE	NO. FAMILIES	M_{r1}	$i_g M_{r1}$	+	i_g	TOTAL
Margined-1	$+M_{r1}/+M_{r1}$	13	333	..	..	..	333
	$i_g M_{r1}/+M_{r1}$	1	59	15	..	..	74
	$i_g+/+M_r$	22	458	21	22	153	654
Normal	$++/++$	1	..	..	47	..	47
	$i_g+/++$	1	..	..	34	10	44
Interaxil-green	i_g+/i_g+	8	..	..	..	148	148

recombination, which proves that HAGIWARA and the author were experimenting with the same genes, interaxil-green and margined–1. The white margin in question can be identified as due to M_{r1} by other linkage data, appearing in the following pages.

Next we shall consider the relation between interaxil-green and intense. The S_1 data of 375–DA, arranged with respect to these genes, are given in table 12.

TABLE 12

S_1 from plant 375–DA, showing linkage of interaxil-green and intense (i).

	+	i_g	i	$i_g\,i$	TOTAL
Observed	53	15	15	4	87

From these figures the frequency of recombination is calculated to be 49.2 percent, nearly free recombination taking place for these two characters. In S_2 of this pedigree the data shown in table 13 were obtained.

TABLE 13

S_2 from plant 375-DA, showing linkage of interaxil-green and intense.

S_1 PARENT	GENOTYPE	FAMILY NO.	+	i_g	i	$i_g\,i$	TOTAL
	+/+ +/+	3 families	43	..	..	..	43
	$+/i_g$ +/+	7 families	200	70	..	..	270
	+/+ +/i	5 families	184	..	59	..	243
Normal	$+/i_g$ +/i	3	20	7	7	2	36
		6	2	1	1	0	4
		9	13	5	8	1	27
		10	25	8	9	2	44
		14	7	3	6	0	16
		18	19	5	8	2	34
		24	35	12	16	4	67
		25	11	6	6	2	25
		28	19	7	7	2	35
		35	15	7	5	0	27
		38	16	8	5	3	32
Interaxil-green	i_g/i_g +/+	2 families	..	13	..	..	13
	i_g/i_g +/i	3 families	..	71	..	23	94
Intense	+/+ i/i	6 families	..	..	94	..	94
	$+/i_g$ i/i	6 families	..	..	113	42	155
Interaxil-green intense	i_g/i_g i/i	3 families	..	..	..	41	41

On account of the weakness of the linkage, definite evidence of which will be shown later, the families segregating dihybridly cannot be clearly separated into coupling and repulsion series. The families in table 13 seems to favor the theory of weak linkage rather than non-linkage.

Finally, let us consider the relation between margined–1 and intense. Table 14 contains the S_1 data of this pedigree.

The frequency of recombination is 48.2 percent. The S_2 data of this pedigree with regard to the segregation of margined–1 and intense are arranged in table 15.

TABLE 14

S_1 from plant 375-DA, showing linkage of margined-1 and intense.

	M_{r1}	M_{r1}/i	+	i	TOTAL
Observed	52	15	16	4	87

Owing to the weak linkage, it is not obvious that the segregation is not independent. A cross between margined–1 and intense gave in F_2 additional evidence of a weak coupling distribution as shown in table 16.

TABLE 15

S_2 from plant 375-DA, showing linkage of margined-1 and intense.

S_1 PARENT	GENOTYPE	FAMILY NO.	M_{r1}	$M_{r1}\,i$	+	i	TOTAL
	$M_{r1}/M_{r1}+/+$	4 families	117	..	..	..	117
	$M_{r1}/M_{r1}+/i$	4 families	150	46	..	..	196
	$M_{r1}/+\ +/+$	6 families	142	..	54	..	196
	$M_{r1}/++/i$	3	20	7	7	2	36
		6	2	1	1	0	4
Margined-1		9	12	8	6	1	27
		14	7	6	3	0	16
		18	19	8	5	2	34
		24	35	15	12	5	67
		25	11	6	6	2	25
		28	17	8	9	1	35
		35	15	4	7	1	27
		38	17	5	7	3	32
Margined-1 intense	$M_{r1}/M_{r1}\ i/i$	6 families	..	94	..	..	94
	$M_{r1}/+\ i/i$	7 families	..	150	..	54	204
Normal	$+/+\ +/+$	2 families	..	..	13	..	13
	$+/+\ +/i$	4 families	..	..	102	34	136
Intense	$+/+\ i/i$	3 families	..	..	..	41	41

From this table the frequency of recombination is estimated to be 43.4 percent, which corresponds with the figure calculated from table 14;

neither value, however, being very dependable on account of the small numbers of individuals.

Thus, the three genes for interaxil-green, margined-1 and intense may be linked to each other. The determination of the order of their loci with these data, however, is more difficult. It will be made later on the basis of other data.

TABLE 16

F_2 from the cross of margined-1 by intense.

CROSS	M_{r1}	M_{r1} i	+	i	TOTAL
59×B16	44	14	16	8	82

A cross was made between a pure stock (No. 500) having green stems, white flowers with white tubes and white seeds (white–3) and another pure strain (No. 65) bearing white-margined intense flowers with colored tubes and black seeds. On selfing the normal F_1 flowers, the writer obtained F_2, which showed linkage of margined-1, tube-white and intense. In table 17 the F_2 data are arranged to show the gene relation between white-tube and intense.

The white tube is clearly distinguishable from the colored tube and its gene is called tube-white and is symbolized as t_w. The white-flowered segregates invariably have green stems, white tubes and white seeds.

TABLE 17

F_2 from cross 500×65, showing linkage of tube-white (t_w) and intense.

	+	t_w	i	t_w i	TOTAL	WHITE-3
Observed	374	167	157	8	706	247

These characters are considered to be manifold effects of a recessive gene w_3 (formerly x). MIYAZAWA (1923), however, recorded 42 colored flowers with black seeds, 2 colored flowers with white seeds, 1 white flower with black seed and 18 white flowers with white seeds amongst 63 plants in an exceptional F_3 family of a cross made between white flower with white seed and white flower with black seed. His conclusion was that white flower and white seed are due to different but closely linked genes. But the records for flower color and for seed character are naturally made at different times, and it is possible that an occassional mistake was made in recording their connection. In the writer's experiments, no such exceptions

were noted. The F_2 data of cross 500×65 give evidence that the white flower (w_3) assorts quite freely with t_w and also with i. Since white–3 does not belong to this linkage group, it can be neglected in the calculation of the linkage value of the other genes, t_w and i. From the figures given in

TABLE 18

F_3 offspring of normal F_2 from cross 500×65, showing linkage of tube-white and intense.

NO. FAMILIES	+	t_w	i	$t_w i$	TOTAL	WHITE–3
4	145	68	62	2	277	..
8	150	75	60	6	291	93
Total	295	143	122	8	568	..

table 17 we estimate the frequency of recombination for t_w and i to be 24.2 percent. In F_3 the writer did not take a complete record on every family, but examined 12 families segregating in regard to the genes t_w and i, the data being collected in table 18.

TABLE 19

F_2 from cross 500×65, showing linkage of margined and tube-white.

	M_r	$M_r t_w$	+	t_w	TOTAL	WHITE–3
Observed	88	0	443	175	706	247

All families show repulsion, giving 24.7 percent recombination. The available data from both generations give 24.6 percent recombination.

Next let us consider the relation between margined and tube-white in the data of cross 500×65. Table 19 shows the F_2 segregation of the characters in question.

TABLE 20

F_3 offspring of margined F_2 from cross 500×65, showing linkage of margined-1 and tube-white.

NO. FAMILIES	M_{r1}	$M_{r1} t_w$	+	t_w	TOTAL	WHITE–3
3	111	0	5	38	154	..
3	43	0	2	12	57	26
Total	154	0	7	50	211	..

The genetics of white margin in this cross has been worked out elsewhere (IMAI 1927a) with the assumption of three dominant genes, M_{r1}, M_{r2} and

M_{r-s} (formerly F^a, F^b and F^h), the first two being formative genes for its production, the white-margin type being a double dominant, and the third one acting as a suppressor. These three genes are supposed to assort freely, not only with each other but also with w_3. From the discussion above, linkage of some degree is expected for the genes M_{r1} and t_w, and seems to exist in the data of table 19. Owing to the genetic complexity of the white margin in this case, however, it is difficult to determine the linkage value directly from these figures. The F_3 was examined for this segregation and the data showing the segregation for the genes M_{r1} and t_w only are given in table 20.

TABLE 21

The theoretical F_2 ratio of margined to normal in cross 500×65, when the effect of tube-white is considered.

GENOTYPE	RATIO	PHENOTYPE	TOTAL
$M_{r1}\ M_{r2}\ M_{r-s}+$	$27x^3+36x+18$		
$+M_{r2}\ M_{r-s}+$	$18x+9$		
$M_{r1}+M_{r-s}+$	$9x^2+12x+6$		
$++M_{r-s}+$	$6x+3$		
$+M_{r2}++$	$6x+3$		
$M_{r1}+++$	$3x^3+4x+2$		
$++++$	$2x+1$		
$M_{r1}\ M_{r2}\ M_{r-s}\ t_w$	$18x+9$	normal	$55x^3+116x+58$
$+M_{r2}\ M_{r-s}\ t_w$	$9x^3$		
$M_{r1}+M_{r-s}\ t_w$	$6x+3$		
$++M_{r-s}\ t_w$	$3x^3$		
$+M_{r2}+t_w$	$3x^3$		
$M_{r1}++t_w$	$2x+1$		
$+++t_w$	x^3		
$M_{r1}\ M_{r2}+t_w$	$6x+3$		
$M_{r1}\ M_r++$	$9x^2+12x+6$	margined	$9x^2+12x+6$

The segregation shows coupling, but there were no white-margined flowers with white tubes. From other experiments it seems clear that the white margin cannot manifest itself in flowers with white tubes (t_w), and this peculiarity is presumed to be due to gene interaction. If this assumption is applied to the data of table 20, the number of white-margined flowers with white tubes may be assumed to be equal to the number of self-colored flowers with colored tubes, that is, 7 in this case, and deducted from the number of double recessive segregates, giving:

M_{r1}	$M_{r1}t_w$	+	t_w	TOTAL
154	7	7	43	211

On the basis of these figures the frequency of recombination is 7.1 percent.

The fact that the white margin cannot develop in flowers with white tubes changes somewhat the F_2 ratio of white-margined and self-colored flowers in this cross. In a former paper (IMAI 1927a), the writer calculated the theoretical F_2 ratio without taking into account the segregation of the white tube. If the gametic ratio in the segregation of the genes M_{r1} and t_w is x:1 (coupling series), the theoretical F_2 of cross 500×65 is shown in table 21 (compare table 21, 1927a).

TABLE 22

F_3 offspring of margined F_2 from cross 500×65, showing linkage of margined-1 and intense.

NO. FAMILIES	M_{r1}	M_{r1} i	+	i	TOTAL	WHITE-3
1	12	4	2	0	18	. . .
2	17	8	5	1	31	14
Total	29	12	7	1	49	. .

When x is replaced by 13, the value corresponding to 7.1 percent recombination, the ratio of white-margined to self-colored flowers becomes 10861:1683; that is, 6.5:1, instead of 55:9 or 6.1:1 as formerly estimated. Actual F_2 of this cross was composed of 88 white-margined and 618 self-colored flowers, where, according to the new view, we expect 95 and 611 respectively.

TABLE 23

F_2 from HAGIWARI, showing linkage of margined-1, and intense.

CROSS	M_{r1}	M_{r1} i	+	i	TOTAL
54×43	218	58	49	39	364
72×53	68	17	21	18	124
56×72	85	16	18	21	140
60×72	21	6	5	7	39
Total	392	97	93	85	667

The former data in regard to linkage of M_{r1} and i (see tables 14 and 16) gave 48.2 percent recombination from the repulsion series and 43.4 percent from the coupling series; these figures, however, may not indicate precise values because the data on which the calculation was based were not sufficient in number.

According to the results already examined in the hybrid progeny of cross 500×65, the frequency of recombination for t_w and i is 24.6 percent and that for M_{r1} and t_w is 7.1 percent; therefore, for M_{r1} and i we may expect about 31.7 percent (7.1+24.6) or about 17.5 percent (24.6–7.1). The value 31.7 is in better agreement with the values (48.2, 43.4) calculated from the experiment, and hence we may conclude that the order of genes is M_{r1}—t_w—i.

TABLE 24

S_1 expectation for segregation of margined-1, tube-white and margined-2 in pedigree M129.

GENOTYPE	RATIO	PHENOTYPE	TOTAL
M_{r1} M_{r2}+	$9x^2+12x+6$	margined	$9x^2+12x+6$
M_{r1}++ +M_{r2}+ +++	$3x^2+4x+2$ $6x+3$ $2x+1$	normal	$3x^2+12x+6$
M_{r1} M_{r2} t_w M_{r1}+t_w +M_{r2} t_w ++t_w	$6x+3$ $2x+1$ $3x^2$ x^2	tube-white	$4x^2+8x+4$

For F_3, the majority of families were bred from self-colored F_2 plants. Only 3 families propagated from white-margined F_2 flowers segregated for M_{r1} and i only. The data are shown in table 22.

The number is too small to determine definitely the linkage value at issue. However, HAGIWARA'S data (1922b), which are collected in table 23, fix this linkage value.

TABLE 25

S_1 from plant M129, showing segregation of margined-1, tube-white and margined-2.

	M_r	$M_r t_w$	+	t_w	TOTAL
Observed	84	0	28	32	144
Expected	77.28	0	30.72	36	144

$\chi^2=1.27$ $P=0.54$

The calculation of the frequency of recombination shows 32.4 percent. This value is very close to the expected 31.7 percent. Thus, it is possible for us to decide definitely the mutual relationship of these three genes; their order is M_{r1}—t_w—i.

On selfing a "parental stock", No. M129, bearing white margined flowers the writer bred generations S_1 and S_2. With respect to a_c, M_{r2}, c_t and M_{r1} the behavior and linkage were practically the same as previously reported (IMAI 1927a). The strain was heterozygous for both M_{r1} and M_{r2}, which

TABLE 26

S_1 from plant M129, showing segregation of margined-1, tube-white and margined-2.

S_1 PARENT	GENOTYPE	NO. FAMILIES	M_r	$M_r t_w$	+	t_w	TOTAL
Margined	$\frac{M_{r1}+}{M_{r1}+}\ \frac{M_{r2}}{M_{r2}}$	1	10	..	..	..	10
	$\frac{M_{r1}+}{M_{r1}+}\ \frac{M_{r2}}{+}$	2	30	..	14	..	44
	$\frac{M_{r1}+}{+\,t_w}\ \frac{M_{r2}}{+}$	11	241	0	109	127	477
	$\frac{M_{r1}+}{+\,t_w}\ \frac{M_{r2}}{M_{r2}}$	1	20	0	0	6	32
Normal	?	1	..	..	30	..	30
	?	3	..	..	19	7	26
Tube-white	?	4	..	..	..	55	55

work complementaly for the production of the white-margin character. M_{r1} is linked very closely with c_t. M_{r2} gives strong linkage with a_c. Let us now consider the other relations involved in this cross. Since the linkage of M_{r1} and t_w was of the coupling type, the genetic constitution of the mother

TABLE 27

S_1 from plant M129, showing linkage of contracted (c_t) and tube-white.

	+	c_t	t_w	$c_t\ t_w$	TOTAL
Observed	75	33	36	0	144

plant of this pedigree was $M_{r1}+/+t_w\ M_{r2}/+$. On selfing such a triply heterozygous plant, we should expect the offspring shown in table 24.

If we substitute 13 for x in the above formula, the expectation is 1683 margined: 0 margined tube-white: 669 standard type: 784 tube-white. In S_1 generation, the writer obtained the segregation shown in table 25.

Thus, the observed result agrees quite well with the expectation.

Next, in table 26, we may present the S_2 data of pedigree M129, in relation to genes, M_{r1}, t_w and M_{r2}.

According to the theory, we should expect two types of segregation in the families, from the white-margined, magenta-tubed parents, one corresponding to $+t_w/M_{r1}+\ +/M_{r2}$ and the other to $+t_w/M_{r1}+M_{r2}/M_{r2}$. Eleven

TABLE 28

S_2 from plant M129, showing linkage of contracted and tube-white.

S_1 PARENT	GENOTYPE	NO. FAMILIES	+	c_t	t_w	$c_t\ t_w$	TOTAL
Normal	++/++	1	10	..	..	..	10
	c_t+/++	2	34	10	..	..	44
	+t_w/++	9	275	..	99	..	374
	c_t+/+t_w	5	84	34	35	1	154
Contracted	c_t+/c_t+	1	..	30	..	..	30
	$c_t\ t_w/c_t$+	1	..	5	..	2	7
Tube-white	$c_t\ t_w$/+t_w	3	..	..	35	15	50
Contracted tube-white	$c_t\ t_w/c_t\ t_w$	1	..	..	..	5	5

families were found segregating in the former fashion, whereas one family segregated in the latter fashion. If x:1 is substituted for the coupling gametic ratio, in the latter case, the ratio expected is:

Margined	$3x^2+4x+2$
Margined tube-white	0
Standard type	$2x+1$
Tube-white	x^2+2x+1

If x = 13, the proportion expected is 561:0:27:196 for the four forms.

Next let us make an analysis of the relation of contracted and tube-white. The S_1 data of pedigree M129, arranged for this purpose, are given in table 27, and the S_2 data in table 28.

In table 28, five families segregated dihybridly and showed repulsion of c_t and t_w. The total, plus that obtained in the foregoing generation, is:

+	c_t	t_w	$c_t t_w$	TOTAL
159	67	71	1	298

On this basis the frequency of recombination is estimated to be 18.4 percent. This result, however, is not very reliable because in calculating

repulsion the value depend very much upon the small number of the double recessive segregates. As shown in the foregoing pages, the frequency of recombination for c_t and M_{r1} is 1.0 percent and that between M_{r1} and t_w is 7.1 percent, so the expected linkage frequency for c_t and t_w is either 8.1 percent (1.0+7.1) or 6.1 percent (7.1−1.0). Another method of calculating the linkage value is available in this case. From the segregation in 23 families it is possible to determine the origin of the gametes which contributed to their production. They are as follows:

Original combinations	42
Recombinations	4

From these figures the frequency of recombination is calculated as 8.7 percent. This result is very close to the expected frequency, namely, 8.1 percent. Therefore, the order of these three genes in the chromosome may be assumed to be c_t—M_{r1}—t_w; that is, the c_t locus is probably situated to the left of the M_{r1} locus.

TABLE 29

Data on linkage of white-2 and dragonfly, from HAGIWARA.

SOURCE	+	w_2	d_g	$w_2 d_g$	TOTAL
Table 1	438	79	72	73	662
Table 9	305	56	58	92	511
Total	743	135	130	165	1173

HAGIWARA (1922b, 1926a) demonstrated linkage between white flower and dragonfly leaf. In table 29, the writer has collected from HAGIWARA's paper (1926a) the available data bearing on this relation.

With these figures, TAKEZAKI's formula gives 25.7 percent recombination. According to HAGIWARA (1922b), white flower is linked closely with intense flower color. Prior to this paper the existence of linkage of these characters was pointed out by the writer (IMAI 1921b) on data published by MIYAZAWA (1920), who attributed the particular behavior to gene interaction. There are three or more kinds of recessive white flowers, genetically quite different. One of these, white–3, has already been refered to. It has white flowers with white tubes, green stems and white seeds. Another, white–2, has white flowers with colored or white tubes, green stems and black seeds. The remaining white flower, white-1, has white tubes, colored stems and black seeds. The double recessive $w_1 w_2$ has white flowers with white tubes, green stems and black seeds. The white flower which is linked with dragon-

fly leaf and intense color is white-2. The writer tentatively calculated the frequency of recombination for i and w_2 on the basis of MIYAZAWA's data and found less than 1.4 percent. Acording to HAGIWARA (1922b), the frequency of recombination for intense (i) and dragonfly (d_g, formerly k) is 40 percent. It follows that the order of these three genes in the chromosome is probably i—w_2—d_g. The fit of the value 40 with the higher expectation (1.4+25.7) is not very close, but, at present, we have no critical data to determine the value more accurately.

HAGIWARA (1922b) showed the relations of d_g to c_t and M_{r1}. According to his calculation the recombination frequency for c_t and d_g is 41.7 percent and that for M_{r1} and d_g is 38.5 percent. This suggests that the distance of the d_g locus from c_t is a little longer than from M_{r1}. The order indicated for these genes is thus: c_t—M_{r1}—d_g.

The writer (IMAI 1927b) detected a reducer M_{r-r} (formerly F^p) for the white margin, linked to intense with 3.0 percent recombination. The locus of this gene has not yet been determined with relation to any second locus in the chromosome.

We are now prepared to draw conclusions concerning the contracted linkage group and to present a preliminary chromosome map. From the foregoing calculations, with utilization of HAGIWARA's data, we found eight genes linked together, that is, i_g, c_t, M_{r1}, t_w, i, w_2, M_{r-r} and d_g. The relative positions of their loci in the chromosome, approximately determined on the basis of the data given above, are diagrammatically indicated in figure 1 partly verifying and extending HAGIWARA's results.

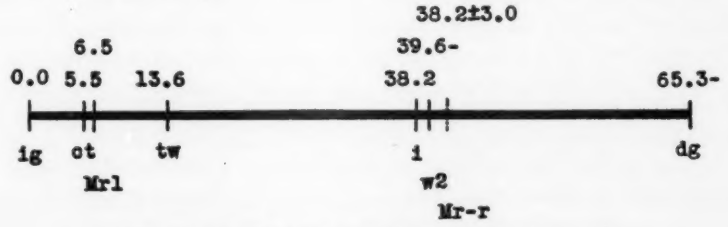

FIGURE 1.—Preliminary map of the contracted chromosome.

SPECKLED LINKAGE GROUP

In the F_2 and F_3 progeny of a cross, the writer detected two genes for the distribution of flower color, linked to each other very closely. Owing to the complexity of the data some preparatory description is necessary before entering on the main discussion. The cross was between a white-

flowered strain (No. 3) and a speckled strain (No. 350). The white-flowered strain was white-1 (w_1), which has stems that are self-colored. The speckled strain had the intense blue color of the flowers restricted to small spots or speckles by the occurrence of much yellowish white on the flower as a back-ground for the speckles. Similarly, the stems of the speckled strain had the normal dark magenta color restricted to spots by the appearance of a green back-ground.

The F_1 hybrid had normal self-colored stems and bore normal self-colored flowers. An observation on the F_2 seedlings showed the segregation indicated in table 30.

TABLE 30

F_2 from cross 3×350, showing segregation for character of hypocotyl of the seedlings.

	+	s_p	$s_p s_{p-r}$	TOTAL
Observed	88	16	8	112
Expected	84	21	7	112

$\chi^2=1.52$ $P=0.48$

Three hypocotyl forms, namely self-colored, speckled and a new form, appeared in the proportion of 12:3:1. The new form was speckled, but the spots were reduced very much in size and in numbers so that the stem was nearly pure green. A satisfactory explanation of the new type is given by the assumption of a second gene, which reduces still further the color of stems and flowers. This gene, which we may call speckled-reducer (s_{p-r}), acts as an intensifier of the action of the speckled gene. The nearly green stem is the double recessive, $s_p\ s_{p-r}$. The speckled-reducer could not have been in the speckled strain, and must have been brought into the cross by the white-1 parent, which was a homozygous double recessive for w_1 and s_{p-r}. The speckled-reducer gave no apparent effect upon the color of stems of the white-1 strain. Therefore speckled-reducer may be considered a specific modifier of speckled. If there is no linkage of speckled and speckled-reducer the F_2 ratio would thus be expected to be 12+: 3 s_p: 1 s_p s_{p-r}, and actually was nearly this.

The seedlings recorded in table 30 were grown until they flowered and were again classified, with the results given in table 31.

The eight speckled speckled-reduced F_2 plants had flowers with the yellowish back-ground much more extensive than in the simple speckled parental strain and in the 16 F_2 speckled plants. But the spots of color

remaining on the flowers of speckled-reduced plants were a little larger and more numerous than the corresponding spots on the stems, where they were very hard to find. The 88 plants with colored stems gave 59 plants

TABLE 31

F_2 from cross 3×350, showing segregation for the flower color.

	+	w_1	s_p	$s_p s_{p-r}$	TOTAL
Observed	59	29	16	8	112

with normal flowers and 29 plants with white flowers. There were no plants with white flowers and speckled or speckled-reduced stems. This situation can be interpreted as very strong repulsion of speckled (s_p) and white-1 (w_1). The F_2 ratio is thus nearly $8+: 4\ w_1: 3\ s_p: 1\ s_p\ s_{p-r}$.

F_3 families were bred from F_2 plants of the four phenotypes and are given in table 32.

TABLE 32

F_3 from cross 3×350, showing segregation for the flower color.

F_2 PARENT	GENOTYPE	NO. FAMILIES	+	w_1	s_p	$s_p\ s_{p-r}$	$w_1\ s_p\ s_{p-r}$	TOTAL
	$\frac{w_1+}{+s_p}$ $\frac{+}{+}$	13	108	63	45	..	..	216
	$\frac{w_1+}{+s_p}$ $\frac{s_{p-r}}{s_{p-r}}$	9	103	54	..	60	..	217
Normal*	$\frac{w_1+}{+s_p}$ $\frac{s_{p-r}}{+}$	9	138	81	58	22	..	299
White-1	$\frac{w_1+}{w_1+}$?	20	..	389	..	..	..	389
Speckled	$\frac{+s_p}{+s_p}$ $\frac{s_{p-r}}{+}$	5	..	..	59	29	..	88
Speckled speckled-reduced	$\frac{+s_p}{+s_p}$ $\frac{s_{p-r}}{s_{p-r}}$	4	..	..	..	105	..	105
	$\frac{w_1 s_p}{+s_p}$ $\frac{s_{p-r}}{s_{p-r}}$	1	..	..	..	7	3	10

* Owing to the small number of individuals contained, five families are omitted from this table.

Of the normal plants, 9 repeated the F_2 results. Nine others were heterozygous for speckled and white-1 and were homozygous for speckled-reducer. The remaining 13 were heterozygous for speckled and white-1 and were free from speckled-reducer.

Of the 5 speckled-reduced plants, one was heterozygous for white-1. This F_2 plant must have resulted from crossing over, which allowed both speckled and white-1 to be carried by the same chromosome strand between loci s_p and w_1. The entire F_3 gave 61 plants, coming from 122 gametes. Of these gametes, only 1, or 0.8 percent, resulted from crossing over.

The writer (IMAI 1927a) has shown that a fluctuator for white margin, M_{r-f}, is linked with white-1, with about 20 percent recombination. The speckled linkage group therefore contains three genes, s_p, w_1 and M_{r-f}.

DELICATE LINKAGE GROUP

The "Sasa" leaf is due to a recessive gene called delicate, d_1, which works as a leaf-form modifier. The gene d_1 results in split flower, in addition to the particular leaf modification. So far as we are aware, only one gene linked with d_1 has been detected. A crumpling of the leaf is brought about by either one of two genes, termed crumpled-1 (c_1) and crumpled-2 (c_2) respectively. The former, crumpled-1, as described on an earlier page, is linked very closely with variegated; and the latter, crumpled-2 shows strong linkage with delicate. The frequency of recombination for d_1 and c_2 was calculated to be about 5 percent (IMAI 1926). No further observations have been made.

PEAR LINKAGE GROUP

It is well-known to cultivators of the Japanese morning glory that fasciated specimens nearly always bear pear leaves. HAGIWARA (1924, 1926c) suggested a two-gene hypothesis to account for his results on fasciation and he regarded one of the two recessive genes for the formation of fasciation as responsible for pear leaf also. YAMAGUCHI (1926), however, gained evidence for breakage between fasciation and pear leaf, and he concluded that the frequency of recombination for pear and one of the two fasciation genes is about 4 percent. The writer (IMAI 1927c) also obtained data for the breakage of the linkage of pear leaf and fasciated stem; but he explained his results on fasciation by a three-gene hypothesis. According to the writer's opinion, fasciation is a triple recessive form, homozygous for f_1, f_2 and f_3. The first two genes are linked with about 20-25 percent recombination. The marked accompaniment of pear leaf and fasciated stem is due to linkage of p (pear) with f_1 and f_2, with about 2.5 percent recombination for p and f_1.

Studying the genetics of leaf form, the writer (IMAI, in press) found two dominant and one recessive genes, each of which produced a blown character. On the basis of repulsion data collected by the writer, one of these dominants, B_2, which results in a weakly characterised blown condition, is linked with pear, with about 23.5 percent recombination.

RAYED LINKAGE GROUP

A pedigree strain, No. 115, bears cream flowers with a few fine dots which are dilute magenta in color. The author studied the ever-sporting behavior of this strain, which gives self-colored plants in its progeny (IMAI 1927d). A cream-flowered plant may also bear, though not frequently, a fully colored flower, which is due to somatic variation. The hybridization of this strain with M_4 and A_4, pure stocks bearing the normal self-colored flowers, gave F_1 plants with colored flowers. But the color was not distributed uniformly on the corollas, the color of the rays being faded nearly to the midrib region. Such a pattern of coloration will be called rayed. The rayed hybrids gave F_2, segregating as shown in table 33.

TABLE 33

F_2 from the cross of cream by normal, showing linkage of rayed (R_y) and cream (c_r).

CROSS	R_y	+	c_r	TOTAL
115×M4	60	20	17	97
115×A4	65	35	24	124
Total	125	55	41	221

TABLE 34

F_3 offspring of rayed F_2, from cross 115×A4.

NO. FAMILIES	R_y	+	c_r	TOTAL
29	379	210	185	774
1	22	7	..	29

The segregation of the three F_2 forms, rayed, normal and cream, occurred in approximately a 2:1:1 ratio. Eighty-nine F_3 families of cross 115×*A*4 were raised to determine the genetic behavior, and the record of these was taken. The 89 F_2 plants, from which F_3 families were bred, consisted of 37 rayed, 30 normals and 22 creams. If we omit the data of families, Nos. 1,

44, 53, 60, 61, 81 and 83, which contain very few offspring, the rayed F_2 plants gave the F_3 results shown in table 34.

The manner of segregation is practically the same as in the previous generation, 29 families repeating the ratio 2 rayed: 1 normal: 1 cream. One exceptional family (No. 64) gave only rayed and normal flowers, with

TABLE 35

F_3 offspring of cream F_2, from cross 115×A4.

NO. FAMILIES	R_p	+	c_r	TOTAL
22	25	1	532	558

no creams. Before stating the significance of this exceptional family it is advisable to present the other data on the offspring of normal and cream F_2 plants. The normal F_2, plants without exception, bred true to type; 30 families included 524 plants, all of which bore self-colored flowers. The cream F_2 plants gave some not-cream mutants among the cream offspring (see table 15, IMAI 1927d). A summary of the data of 22 cream F_3 families is given in table 35.

TABLE 36

S_1 from two "parental stocks," showing linkage of duplicated (d_p), striped (s_l) and dilute (D).

PEDIGREE	D	+	$s_l D$	s_l	$d_p D$	d_p	$d_p s_l D$	$d_p s_l$	TOTAL
S371	61	4	1	6	6	0	3	14	95
S373	89	8	1	9	8	0	7	22	144
Total	150	12	2	15	14	0	10	36	239

Among 558 cream F_3 plants, 532 bore cream flowers; the others, which bore not-cream flowers, are considered to have been produced by mutation. Of 26 mutants, 25 had rayed flowers and 1 had normal flowers. The latter appeared as a single mutant plant in family No. 87. The results so far gained are peculiar in some respects in regard to the segregation of the flower color. First, the segregation of the three forms, rayed, normal and cream, occurred nearly in a 2:1:1 ratio. Second, all rayed F_2 plants, with one exception, gave rise to three forms in their offspring and not one rayed plant bred true to type. Third, all normal F_2 plants bred true to the uniformly colored condition. Forth, all not-cream mutants, which appeared among the offspring of cream F_2 flowers, were of rayed flowers, excepting

one case of self-colored flower. These peculiarities are reasonably solved by the assumption of high linkage of the rayed and cream genes. Now let us designate the gene for cream by c_r, and that for rayed by R_y. The gene R_y, however, apparently cannot manifest its effect in the cream flower. The parental genotypes are considered to be homozygous normal for M_4 and A_4, because they bore self-colored flowers, and $R_y\ c_r$ for 115, because it bore cream flowers and produced at times rayed-flowered mutants in its

TABLE 37

S_2 offspring of dilute S_1 from plant S 371, showing linkage of duplicated, striped and dilute.

S_1 PARENT	REFERENCE	NO. FAMILIES	D	+	$s_t\ D$	s_t	$d_p\ D$	d_p	$d_p\ s_t\ D$	$d_p\ s_t$	TOTAL
$\frac{++D}{++D}$	a	2	74	..	..	..	..	..	..	..	74
$\frac{++D}{+++}$	b	2	29	10	..	..	..	..	..	..	39
$\frac{+s_t+}{++D}$	c	3	75	5	4	20	..	..	..	..	104
$\frac{d_p+D}{+++}$	d	1	19	6	..	..	5	0	..	..	30
$\frac{d_p\ s_t+}{++D}$	e	1	20	3	0	4	4	0	1	4	36
$\frac{d_p\ s_t\ D}{+++}$	f	2	32	11	2	0	1	0	10	0	56

offspring. Crosses of these strains resulted in doubly heterozygous F_1 plants bearing rayed flowers. These should give rise to F_2 consisting of 9 rayed, 3 normal and 4 cream plants for every sixteen, if the assortment of genes occurs freely. Actually, however, the segregation is much nearer to 2:1:1 than to 9:3:4. Let us assume that a nearly complete repulsion has taken place, resulting in the ratio observed in F_2. The intensity of linkage, however, cannot be accurately determined from such data. There are indirect ways of calculating the recombination frequency. If the F_3 families which contain small numbers of individuals (less than ten) are omitted, rayed and normal F_2 parents tested number 27 and 15, respectively. If linkage is complete the rayed plants are expected to carry a $R_y\ c_r/+\ +$ chromosome set, and segregation results in a proportion of 2 rayed:

1 normal: 1 rayed cream. However, if the experiment is carried out on a large scale and linkage is not complete but is very high we may have some rayed F_2 plants carrying a crossover chromosome of the constitution R_y+ or $+c_r$. Such a rayed plant should give progeny segregating in a ratio of 3 rayed: 1 normal or 3 rayed: 1 cream. Examination showed that

TABLE 38

Data on linkage of duplicated and striped, from tables 36 and 37.

SOURCE	+	d_p	s_t	$d_p s_t$	TOTAL
Table 36 S371	65	6	7	17	95
Table 36 S373	97	8	10	29	144
Table 37, e	23	4	4	5	36
Table 37, f	43	1	2	10	56
Total	228	19	23	61	331

of 27 rayed F_3 families, 26 were from $R_y\ c_r/+\ +$ plants and 1 was from $R_y+/+\ +$. All 15 F_3 families, whose parents were normal, bred true to type. This fact means that in all the normal F_2 plants both chromosome were non-crossovers. In the total of 84 gametes, there were 83 chromosomes carrying an original combination of the genes and only one carrying a recombinational chromosome, corresponding to 1.2 percent of recombination.

TABLE 39

Data on linkage of duplicated and dilute, from tables 36 and 37.

SOURCE	D	+	$d_p D$	d_p	TOTAL
Table 36 S371	62	10	9	14	95
Table 36 S373	90	17	15	22	144
Table 37, e	20	7	5	4	36
Total	172	34	29	40	275

The mutants which appeared in the F_3 families from cream parents also furnish data in the intensity of linkage. If linkage is complete all F_2 creams should carry the $R_y\ c_r/R_y\ c_r$ genotype, whereas if it is not complete but very high we may detect, though not frequently, cream flowers of the constitution $+\ c_r/R_y\ c_r$, the upper chromosome having been produced by crossing over. The constitution with respect to the rayed character is concealed in the cream flowers, but mutation to not-cream allows the rayed

or the normal character to show. In the $R_y\ c_r/R_y\ c_r$ families, the mutants invariably bear rayed flowers. In the $+c_r/R_y\ c_r$ families, however, one quarter of the F_2 mutants should be non-rayed. As was shown in table 35, one normal mutant was obtained with 25 rayed mutants. The low proportion of normal to rayed mutants also indicates linkage.

TABLE 40

Repulsion data for segregation of duplicated and dilute, from table 37.

SOURCE	D	+	$d_p D$	d_p	TOTAL
Table 37, d	19	6	5	0	30
Table 37, f	34	11	11	0	56
Total	53	17	16	0	86

DUPLICATED LINKAGE GROUP

Several double flowers in this plant have been studied genetically (MIYAKE and IMAI, 1927). Among them, the recessive duplicated type ("Botan') is rather common in gardens. The duplicated flower has no sexual organs, these being replaced by flower buds. Owing to their absence, this flower is obtained only by segregation from the heterozygous plants called "parental stocks".

TABLE 41

Data on linkage of striped and dilute, from tables 36 and 37.

SOURCE	D	s_t	+	$s_t D$	TOTAL
Table 36 S371	67	4	4	20	95
Table 36 S373	97	8	8	31	144
Table 37, c	75	4	5	20	104
Table 37, e	24	1	3	8	36
Total	263	17	20	79	379

On selfing two "parental stocks", Nos. S371 and S373, of unknown origin, the writer bred progenies showing linked segregation as regards three flower characters, duplicated (d_p, formerly b_t), the dominant dilute[1] (D) and the recessive striped (s_t). The data for these are shown in table 36.

[1] For convenience in nomenclature, the intensity of flower color previously termed "dilute" is taken as standard or normal, regarding "intense" and "dilute" as modifications in opposite directions from this normal.

We readily see that the three genes, d_p, s_t and D, did not assort independently, but were linked. The chromosomal constitution of the mother plants is considered to be $d_p\ s_t+/++D$. Before attempting the calculation of the linkage values the writer presents the data of the S_2 generation of pedigree S371 as given in table 37.

Let us first calculate the frequency of recombination for the genes d_p and s_t. In table 38 the data for this calculation is summarized from tables 36 and 37.

The calculation shows 13.7 percent recombination for the genes d_p and s_t.

For the relation between d_p and D the available data are summarized in table 39.

TABLE 42

Repulsion data for segregation of striped and dilute, from table 37.

SOURCE	D	s_t	+	$s_t D$	TOTAL
Table 37, f	33	12	11	0	56

From these data the percentage of recombination is 25.6.

In table 37, classes d and f contain repulsion data for duplicated and dilute, as shown in table 40.

Lastly we shall determine the percentage of recombination for the third combination, s_t and D. Table 41 contains the data available on this relation.

On the basis of table 41 the recombination frequency is 10.1 percent.

Two families of table 37 show repulsion segregation for the genes s_t and D. The data are shown in table 42.

The 10.1 percent of linkage naturally explains the absence of double recessives in the small number under observation.

These three genes d_p, s_t and D,constitute a linkage group and their loci are considered to be in the same chromosome, the order being d_p—s_t—D, with 13.7 units between d_p and s_t and 10.1 units between s_t and D.

CONCLUSION

The chromosome number of the Japanese morning glory was first studied by OHGA (ISHIKAWA 1917), but he failed to count the exact number reporting 12-14 in the haploid condition. The writer, examining sections of young root tips of this plant, found probably 28 chromosomes. Therefore the haploid number is probably 14. Although the Japanese morning

glory exhibits numberless variations which are available for genetic investigation, because of the large number of chromosomes it is not an easy matter to complete the chromosome maps, so beautifully studied in Drosophila. In the present paper, the writer describes ten linkage groups in this plant. These groups contain the following genes, in the serial order along the chromosome, as far as known:

(1) Variegated linkage group	v, c_1, B_1 and f_3 (order uncertain).
(2) Cordate linkage group	c_o, f_e and s_c.
(3) Yellow linkage group	y and d_y.
(4) Acuminate linkage group	a_c, $\pm M_{r2}$ and m_g.
(5) Contracted linkage group	i_g, c_4, M_{r1}, l_w, i, w_2, M_{r-r} and d_g.
(6) Speckled linkage group	s_p, w_1 and M_{r-f} (or M_{r-f}, s_p and w_1).
(7) Delicate linkage group	d_e and c_2.
(8) Pear linkage group	p, f_1, f_2 and B_2 (order uncertain).
(9) Rayed linkage group	R_y and c_7.
(10) Duplicated linkage group	d_p, s_t and D.

These ten linkage group contain 34 genes. Up to the present nearly one hundred genes have been detected in this plant. Further investigation should determine the four other linkage groups not yet established, and, on the other hand, it may enable us to combine two or more of these groups into one by finding a linkage relation between them. In addition, some cases of unequal frequency of gene recombination have been recorded, but these should not be reported, in the writer's judgment, until they are definitely established.

SUMMARY

1. Ten linkage groups are established, including 34 loci. Up to the present time, nearly one hundred genes have been detected in this plant.

2. The variegated linkage group contains four genes, v (variegated), c_1 (crumpled-1), B_1 (blown-1) and f_3 (fasciated-3). The percentage of recombination is 17.7 for v and c_1, 29.3 for v and B_1 and about 20-25 for v and f_3.

3. The cordate linkage group includes three genes, c_o (cordate), f_e (feathered) and s_c (semi-contracted). The recombination percent for c_o and f_e is 1.2 (MIYAKE and IMAI 1920) and s_c is located 18.6 units from c_o. The interval between f_e and s_c is estimated to be 17.1. Therefore the arrangement of these genes on the cordate chromosome is probably c_o—f_e—s_c, with a distance of 1.2 between c_o and f_e and 17.1 between f_e and s_c.

4. The yellow linkage group includes two genes, y (yellow) and d_y (dusky), the frequency of recombination for them being 1.0 percent.

5. The acuminate linkage group contains three genes a_c (acuminate), M_{r2}(margined-2) and m_g (magenta). The percentage of recombination is 0.5 for a_c and M_{r2} and 21.2 for a_c and m_g.

6. The contracted linkage group is the largest group, containing eight genes i_g (interaxil-green), c_t (contracted), M_{r1} (margined-1), t_w (tube-white), i (intense), w_2 (white-2), M_{r-r} (margined-reducer) and d_g (dragon-fly). The arrangement of this group is in part due to HAGIWARA'S data (1922b, 1926a). The following order of these genes in the chromosome is deduced: i_g—c_t—M_{r1}—t_w—i—w_2—d_g; if the i_g locus is at 0, c_t is 5.5, M_{r1} 6.5, t_w 13.6, i 38.2, w_2 39.6- and d_g 65.3-; and M_{r-r} is located at 38.2 ± 3.0.

7. The speckled linkage group includes three genes s_p (speckled), w_1 (white-1) and M_{r-f} (margined-fluctuator). Among these, s_p and w_1 are linked very closely, with 0.8 percent of recombination, and linkage of w_1 and M_{r-f} gives about 20 percent of recombination.

8. The delicate linkage group contains two genes d_e (delicate) and c_2 (crumpled-2), the recombination frequency for them being about 5 percent.

9. The pear linkage group includes four genes, p (pear), f_1(fasciated-1), f_2 (fasciated-2) and B_2 (blown-2). The frequency of recombination is about 20-25 percent for f_1 and f_2, about 2.5 percent for p and f_1 and 23.5 for p and B_2.

10. The rayed linkage group includes two genes R_y (rayed) and c_r (cream), the percentage of recombination being 1.2.

11. The duplicated linkage group contains three genes d_p (duplicated), s_t (striped) and D (dilute). The frequency of recombination is 13.7 percent for d_p and s_t, 25.6 percent for d_p and D and 10.1 percent for s_t and D. Their order in the chromosome is d_p—s_t—D, with an interval of 13.7 between d_p and s_t and 10.1 between s_t and D.

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TWO FACTORS INFLUENCING FEATHERING IN CHICKENS

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Received July 17, 1928

INTRODUCTION

The material for this paper has been collected in the course of some studies on transplantation of skin in the fowl. The methods and some of the results have been described by DANFORTH and FOSTER (1927). In these experiments skin of the back was transferred from one chick to another within a few days after hatching and its subsequent reactions followed, in some cases, for as much as fifteen months. For present purposes only a relatively short period of early growth will be considered.

Skin grafting has proved especially satisfactory for analyzing the interaction of genetic and other factors in production of form, color and marking of feathers. It has some disadvantages in studies on the rate of feathering in that certain grafts may at any time begin to regress slowly, necessitating careful and frequent scrutiny of all specimens under observation in order to eliminate abnormal cases. It may be, too, that grafting occasionally causes some modification in the growth rate of feathers on apparently normal skin, but by appropriate methods it seems feasible to detect and evaluate such alterations if they occur. Aside from these possible objections, the method seems to be of considerable value in studying the action of growth factors, and this paper is presented partly as a contribution to the technique of such studies.

There are considerable differences in the time at which feathers first appear in young chicks, and also in the rate at which feathers grow after having made their appearance. These differences tend to be associated with breeds, the so-called American and Asiatic classes being in general more or less retarded, while the Mediterranean and other of the smaller breeds are on the whole precocious in this, as in other respects. Sex also is popularly supposed to be a factor in the rate of feathering, females being said to become feathered more rapidly and uniformly than males.

The observations to be reported relate only to the feathers of a restricted area, the back and anterior part of the rump (the "saddle" or "sweep" of the American Standard of Perfection, 1927). This region will be referred to here simply as the "back." The breeds chiefly considered are the white Leghorn, Rhode Island Red, and barred Plymouth Rock.

NORMAL DIFFERENCES IN THE RATE OF FEATHERING

In all the breeds used in the experiments feathers appear on the back later than they do on a number of other parts of the body. Indeed, the regions immediately in front and behind the one selected are usually more advanced. In the rapidly feathering Leghorns there is ordinarily a good number of pin-feathers along the middle of the back by about the thirtieth day after hatching. Many Plymouth Rocks, on the other hand, do not reach a comparable stage until after the fiftieth day. There are rather pronounced individual differences within the same breed, especially among Plymouth Rocks and Rhode Island reds, but the two classes, slow-feathering and rapid-feathering, are reasonably distinct from each other with very few doubtful cases.

The heredity of these two types of feathering has been studied by SEREBROVSKY (1922) and WARREN (1925). The latter found that in crosses involving white Leghorns and Jersey Black Giants (American class) slow-feathering, as indicated by the age at which tail feathers appear, behaves as a dominant sex-linked trait. His conclusion is supported by consistent evidence from reciprocal and back crosses. Earlier results seem to have been fully confirmed by SEREBROVSKY and WASSINA (1926).

To the records already in the literature may be added the numerically meager results of several crosses made in connection with the present experiment. Barred Plymouth Rock females, known to have been slow-feathering, were mated to a white Leghorn male which as a chick had been rapid-feathering. This cross produced three quick-feathering females and a rather slowly feathering male. The outcome, so far as it goes, is in accord with expectations on the basis of the above hypothesis, except that the male was less retarded than is the average Plymouth Rock male at a corresponding age. This is possibly to be explained on the same basis that SPILLMAN (1909) and COLE and LIPPINCOTT (1919) explain differences in width of bars in feathers of the two sexes, namely that, although dominant, the sex-linked gene is more effective in the homozygous male (ZZ) than in the heterozygous female (ZW). It would seem that the suggestion could be tested in both cases by comparing the rate of feathering and width of bars in an adequate number of F_1 offspring from females of a rapid-feathering black breed mated to an ordinary Plymouth Rock male.

Conversely, a slow-feathering Rhode Island Red male mated to white Leghorn hens gave four chicks, all of which were slow-feathering, but not so slow as are many typical Rhode Island Reds and Plymouth Rocks.

Finally, the same Plymouth Rock females and Rhode Island Red male mated together produced six females, all moderately slow in feathering and nine males which were decidedly slow-feathering. The latter, however, showed considerable variation in the time at which the first feathers appeared. At 47 days of age some of them had no feathers or pin-feathers whatever on the back, while others showed a few scattered pin-feathers, and still others feathers with three or four bars.

While in general the foregoing data are in accord with WARREN'S results with Leghorns and Giants and indicate that what is presumably the same dominant sex-linked gene occurs in both the Plymouth Rock and the Rhode Island Red, the diversity found in the F_1 males of Plymouth Rock-Rhode Island Red parentage suggests some additional factor in one or the other of these breeds. The existence of this second factor seems to be established by the results obtained by means of skin grafting.

THE RATE OF FEATHERING ON SKIN TRANSPLANTS BETWEEN RHODE ISLANDS REDS AND WHITE LEGHORNS

In making the transplants, skin from the greater part of the back (as here defined) was taken, most often on the second day after hatching, and sewed in place on the back of the host, from which a piece, preferably somewhat larger, had just been removed. By drenching the backs of chicks to be operated on with alcohol, the use of ether anaesthesia, and reasonable precautions as to asepsis, the procedure was rendered fairly simple. Nevertheless, owing to the loss of many chickens and the failure of some of the grafts on those that lived, the number of individuals that ultimately became available for purposes of the present study was not great. Most of the records were made at weekly intervals, so that dates at which the feathers are first recorded tend to be slightly later than the true dates at which they first appeared. For comparative purposes this is not a matter of importance, since there was no bias in favor of any one group.

Seventeen of the Rhode Island Reds which received white Leghorn grafts survived and produced feathers on the grafted skin. The average age at which the first pin-feathers appeared on the grafts was 33.6 days. This indicates a very slight retardation when compared with the time at which the feathers would have appeared on the Leghorn skin had it not been removed from its original site. In five of these cases both the donors and the hosts were males, and in this group the average age at which pin-feathers appeared was 36 days. In six other cases the donors and hosts were all females and here the average age of pin-feather production

was 32 days. These facts are put on record as suggestive, even though they may not be statistically significant.

The white Leghorn feathers on Rhode Island Red hosts appeared considerably in advance of the host's own feathers and grew more rapidly, so that by the 70th day the grafts bore large white feathers which were surrounded only by small red feathers and pin-feathers. Not until much later did the feathers of the host equal those of the graft in size.

Transplants in the reverse direction presented quite a different picture. Skin from slow-feathering Rhode Island Reds grafted on 23 rapid-feathering white Leghorns showed the first pin-feathers at an average age of 47 days, or about two weeks later than the reciprocal grafts, and after the surrounding feathers of the hosts had become well developed. The average ages of first appearance of pin-feathers with reference to the sex of donors and hosts were as follows: five transplants from male Rhode Island Reds to male Leghorns, 50 days; eight transplants from female to female, 46 days; six from males to females, 46.6 days; three from females to males, 49 days. The sex of one donor was not recorded.

The possibility that the slow feathering of the Rhode Island Red skin grafted on white Leghorns is due to some imperfection of the white Leghorn as a host was tested by eight grafts to white Leghorns from other rapidly feathering strains. These produced feathers at an average age of 32 days, which is comparable to the 33.6 days required for Leghorn skin to produce its feathers when grafted on Rhode Island Reds. This result, in conjunction with the fact that reciprocal grafts between white Leghorns and Rhode Island Reds parallel the unoperated controls in the time at which feathers are produced, seems to leave little room for doubt that in the white Leghorn and Rhode Island Red the genes for slow-feathering and for rapid-feathering act locally in the skin or feather follicles rather than through the intermediation of gonadal or other hormones. At the same time, in both sets there is a suggestion, not adequately supported statistically, that the tendency to slow-feathering is slightly augmented by maleness.

THE RATE OF FEATHERING IN SKIN TRANSPLANTS INVOLVING BARRED PLYMOUTH ROCKS

Barred Plymouth Rock chicks served as donors to chicks of rapidly feathering strains in seven successful cases. The recipients included white and brown Leghorns, black Minorcas and Campines. The average age at which Plymouth Rock feathers appeared on these grafts was 44 days. This agrees very well with what might have been expected in light of

results from the Leghorn-Rhode Island Red combinations. But in the nine cases in which barred Plymouth Rocks served as hosts for grafts from rapidly feathering white Leghorns and Campines, the results were not at all in accord with those obtained in the first series. Here the average age at which the first pin-feathers appeared on the grafts was 47 days. That this late appearance was due to the influence of the hosts and not to the grafts is indicated by results from sixteen cases in which skin was exchanged between other members of these same rapidly feathering breeds,

FIGURE 1.—A barred Plymouth Rock female at the age of 73 days. The white Leghorn graft on its back shows a few pin-feathers whose growth is still considerably inhibited.

the average date of feathering in these cases being 35 days, as would have been expected on the basis of the previous experiments.

This result led to a more critical examination of feather growth in Plymouth Rock chicks and grafts placed upon them. In several cases of grafts to Plymouth Rocks from Campines and Leghorns, the transplanted skin produced a few good feathers early, after which feather-production was seemingly held in abeyance for a while, some of the pin-feathers becoming stunted and even falling out, so that the grafted area assumed the same denuded appearance as other parts of the chick (figure 1). When-

ever any of the first feathers persisted through this period they were of fair size, as they would have been in their normal place, or grafted on a Rhode Island Red, except that some of them were possibly arrested before reaching full development. (see figure 8, a.) During this same period the Plymouth Rock's own feathers appear only sparsely and grow very slowly. In general the whole picture is one suggesting inhibition of feather growth. Later the appearance becomes more normal in both graft and host, the feathers of the former often growing more rapidly at first, after which each type follows its natural course (figure 2).

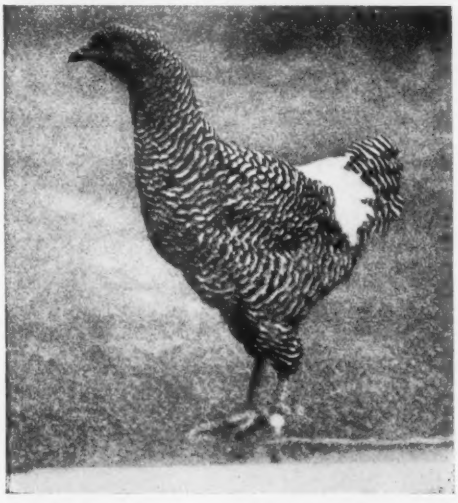

FIGURE 2.—A barred Plymouth Rock pullet with a white Leghorn graft. This is a later stage of the individual shown in figure 1.

Evidence pointing in the same direction is obtained from cases where the Plymouth Rock serves as donor. When the host is a Rhode Island Red the results are not striking, since both donor and host are slow-feathering (figures 3 and 4). But when the host is of a rapidly feathering breed critical comparisons can readily be made between the feathers of the two individuals involved in any transplant. Two such pairs may be reported in some detail.

1. A barred Plymouth Rock graft on a black Minorca host

Barred Plymouth Rock male no. 71 served as donor to black Minorca male (Mediterranean class) no. 74. The two chicks were hatched on the

FIGURE 3.—A Rhode Island Red cockerel with a barred Plymouth Rock graft.

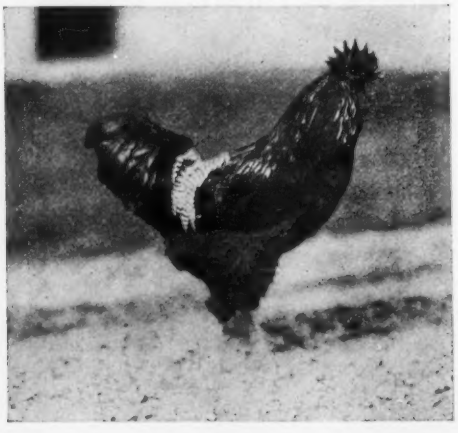

FIGURE 4.—The same bird as shown in figure 3. Both the host and the graft exhibit a parallel development of plumage in this and the preceding figure.

same day and the transplant from the Plymouth Rock to the Minorca was made two days later, enough of the original skin being left on the back of each to produce some feathers characteristic of the individual. Both chicks were kept in the same flock and received similar treatment throughout the experiment. They are shown at the age of 148 days in figures 5 and 6.

No. 71 feathered very slowly but did not pass through a long period in which the body was largely bare, as do many Plymouth Rock males. The Minorca developed his own feathers at the usual rather rapid rate. The graft grew well and showed indications of a few small pin-feathers at the age of 42 days. At 49 days there was one feather. By 75 days the feathers were numerous, most of them showing about three bars.

Representative feathers plucked from the backs of nos. 71 and 74 on the 96th day are shown in the top row of figure 7. The feathers from the grafted skin were larger than those of the donor, but not so large as those of the host. They were slightly constricted, but not of the male type, as perhaps might be inferred from the figures. Feathers of the adult male type began to appear, however, at about this time. The largest of these to be found on the donor at the 110th day is shown as figure 7 d. Figure 7 e, represents, not one of the largest feathers on the graft at this date, but one which, had the skin not been disturbed, would have grown within a few millimeters of 7 d. The bottom row in figure 7 shows two juvenile male feathers from no. 71, and two, somewhat more advanced, from no. 74 plucked at the age of 148 days.

It will be apparent from the records and figures that, while slow as compared with that of the Minorca, the grafted Plymouth Rock skin developed feathers much faster than it would have if it had been left in place. That which remained on the Plymouth Rock showed an initial lag followed by a gradual approach to the rate of growth of that on the graft. Not only the feathers but the whole individual exhibited such a lag. At 96 days no. 71 weighed 820 grams, while no. 74 weighed 1015 grams. At 148 days no. 71 weighed 2080 grams and no. 74 only 1670 grams.

Whether it should be stated that from about the 40th to about the 120th day growth was stimulated by the Minorca soma or inhibited by that of the Plymouth Rock is not evident, but it seems clear that some factor with an origin outside the skin and capable of influencing feather growth was effective during this period.

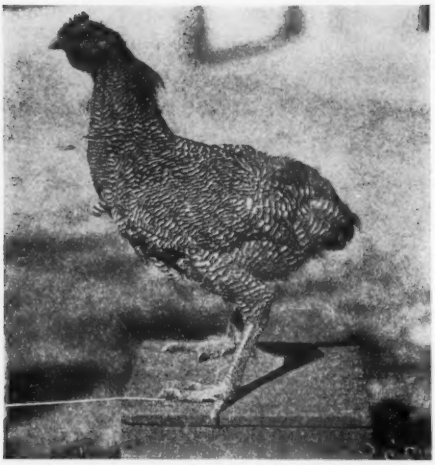

FIGURE 5.—The barred Plymouth Rock male no. 71 at the age of 148 days.

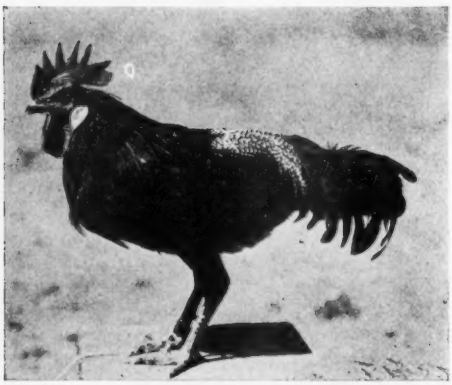

FIGURE 6.—Black Minorca male no. 74 (with graft from no. 71) at the age of 148 days.

2. A barred Plymouth Rock graft on a brown Leghorn host

The treatment and results in this case are similar to those described in the preceding section, except that the graft was from a barred Plymouth Rock male to a brown Leghorn female, and the male himself had a graft from another brown Leghorn female. This male was designated as no. 70, the host female as no. 88.

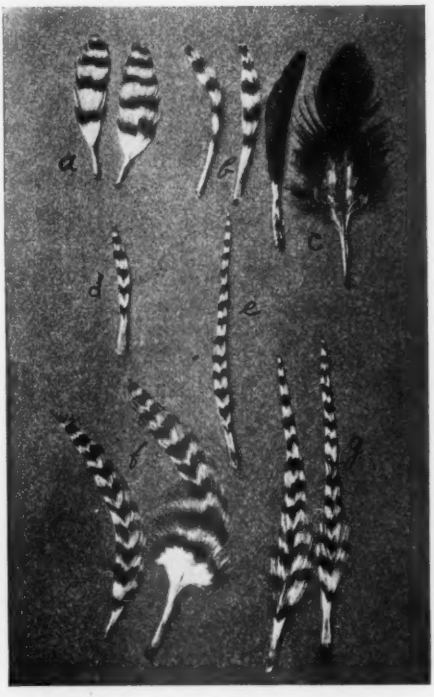

FIGURE 7.—Feathers from nos. 71 and 74 at various ages, arranged transversely according to the age and vertically according to the individual. Top row, 96 days; middle row, 110 days; bottom row, 148 days. a, d, f, feathers from undisturbed skin of no. 71; c, from undisturbed skin of no. 74; b, e, g, from the skin of no. 71 grafted on no. 74.

The graft on no. 70 had produced a few juvenile brown Leghorn feathers by the 34th day, after which it remained more or less quiescent until about the 90th day, during which time the chick as a whole was rather nude. By the 96th day there were many small new feathers like those of a Leghorn male. This is in accord with what is known of the hormonal

control of secondary sexual characteristics in the plumage (GOODALE 1916, DOMM 1927), except that the host at this time had developed no male feathers of his own.

The Plymouth Rock graft (from no. 70) on the brown Leghorn hen no. 88 had one small feather on the 41st day and two on the 48th. By 75 days it had many feathers with five or six bars. These, however, were

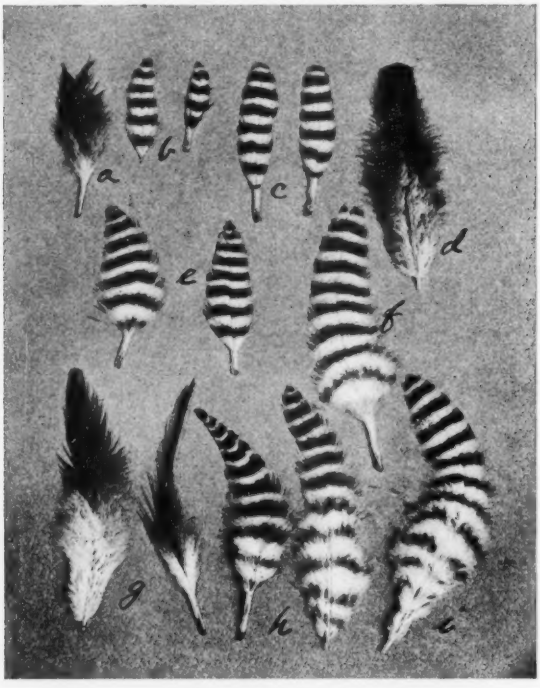

FIGURE 8.—Feathers plucked at different ages from nos. 70 and 88. Top row, 96 days; middle row, 110 days; bottom row, 148 days. a, g, brown Leghorn feathers from the graft on no. 70; b, e, h, feathers from the undisturbed skin of no. 70; c, f, i, from skin of no. 70 grafted on no. 88; d, from the undisturbed skin of no. 88.

recorded as much shorter than those of the host. Figure 8 shows in the top row feathers plucked from no. 70 and no. 88 on the 96th day. Represented by a is one of the residual Leghorn feathers that first appeared on the graft to no. 70. The feathers marked b are the largest that could be found on the back of no. 70 at this time, while those indicated by c are

two average feathers grown on no. 88; *d* is one of 88's own feathers. At the 110th day the discrepancy in size between feathers of the donor, e, and those from the graft, f, is still very great. By the 148th day some of the feathers of the donor, h, have reached a size about equal to that of the grafted feathers, i. Some of the former are now of the juvenile male type.

In this case also the host, again of a rapidly feathering breed, proved to be for a time more favorable for the development of Plymouth Rock feathers than the Plymouth Rock donor itself. During the most of this period the Leghorn graft on the Plymouth Rock was greatly inhibited in its feather production. The period in question appears to extend from as early as the 40th day to well past the 100th day.

The body weight followed the same course in this as in the preceding case. At 96 days no. 70 (male) weighed 810 grams, and no. 88 (female), 990 grams. At 148 days, no. 70 weighed 1680 grams and no. 88, 1350 grams. Similar results were obtained in comparisons between Plymouth Rocks and white Leghorns or Campines.

DISCUSSION

Parallel experiments, where in one case Rhode Island Reds, in the other barred Plymouth Rocks serve as the slow-feathering subjects, show interesting points of similarity and difference. But all the results obtained in both sets appear to be consistent and intelligible on the assumption that there are two different factors making for slow feathering, both present in the barred Plymouth Rock, only one in the strain of Rhode Island Reds used in the experiments. The slow-feathering factor which is common to both breeds is apparently conditioned by the sex-linked gene previously known to exist and designated as *S* by SEREBROVSKY. It is apparent that this gene and its allelomorph, *s*, produce their effects by imparting a particular character to the feather follicles themselves, or to the immediately surrounding tissues, thus determining a definite mode of developmental activity during the first few weeks after hatching. Exchange of skin between two individuals whose genotypes differ in respect to *S* and *s* has no marked effect on the manifestation of these genes. It is the genetic constitution of the donor and not that of the host which determines the rate of feathering in so far as it is regulated by this pair of allelomorphs. This experimental finding is in accord with the observation of WARREN (1925) that in white Leghorns the state of nutrition or physical condition of a chick has very little effect on its rate of feathering.

The other factor is apparently of quite a different nature. In this case it is the constitution of the host and ***not that of the donor*** that exercises

the determining influence in feather growth. In the absence of adequate breeding records nothing can be said of the exact mode of heredity of this factor, or whether it is simple or complex. The most natural inference is that we are concerned here with a hereditary endocrine or metabolic trait. This supposition is strengthened by the parallelism between feather growth and body weight in barred Plymouth Rocks during the critical period under consideration.

The two factors revealed in these experiments are characterized by different modes of expression. The action of one is local and direct, possibly its effect is restricted to the feather follicle. The action of the other is indirect and probably general ("constitutional"). It is of course not improbable that there are other factors of both types which influence the rate of feather production. There is a mere suggestion in the data that sex has a weak effect in both ways; directly, through homo- or heterozygosis, and indirectly through a slight differential effect on the rate of feathering by male and female constitutions. If, as seems not improbable, the second factor is really a combination of several components which happen to be associated in the barred Plymouth Rock, it is possible that more extensive studies will show that certain of these are also present in the Rhode Island Red, but thus far there have been found no indications that this is the case. A few white Plymouth Rocks were observed, and these seemed to agree more closely in their mode of feathering with the Rhode Island Red than with the Barred Plymouth Rock.

It is of passing interest that the factor for indirect production of slow-feathering should appear in one of the races which also has a factor for the direct production of a similar trait. The two factors apparently have no other relation to each other unless there be some as yet undetected endocrine effect in the embryo which sensitizes or imparts some definite character to the skin which determines its future development and responses.

One other point of interest in this connection is the production of cock-feathering on grafts to male hosts. Up to the present time the type of feathering, as regards secondary sexual characteristics, has been believed to be wholly under endocrine control (LILLIE 1927). Nevertheless the graft from a brown Leghorn female to a barred Plymouth Rock male produced cock feathers in advance of the host, and the graft from a Plymouth Rock male to a Minorca male produced cock feathers later than the host but earlier than the donor. Such relations suggest that the production of cock-feathering is in some measure related to the class of phenomena considered in this paper. The time at which a young fowl assumes its adult plumage may depend not alone on the establishment of a certain endocrine balance, but also upon the age at which the feather follicles become re-

sponsive to particular stimuli. Factors for responsiveness to internal stimuli, or for the type of such stimuli produced, may apparently vary independently and become segregated in different strains. (DANFORTH 1928.)

The general inference to be drawn from this study is that activity of a feather follicle, as registered by the feathers which it produces, is regulated by two factors (or sets of factors) which act in quite diverse ways. In genetic or physiologic studies of feather growth both types of factors need to be taken into consideration.

CONCLUSIONS

Transplantation of skin from one newly hatched chick to another has proved to be of value in analysis of factors influencing the rate of feathering.

Evidence obtained by this method indicates that there are two entirely different factors which are capable of producing "slow-feathering" in the young chick.

One of these factors appears as a sex-linked hereditary trait which manifests itself through a direct effect of the gene on the feather follicles.

The other factor produces an indirect inhibitory effect through the intermediation of the soma.

The first of these factors occurs in the Rhode Island Red; both of them are present in the barred Plymouth Rock.

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EFFECTS OF WAXY AND SUGARY GENES ON RESERVES WITH SPECIAL REFERENCE TO MODIFICATION OF THE WAXY RATIO IN MAIZE[1]

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Received July 20, 1928

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INTRODUCTION

The mechanism by which the sugary gene in maize operates to modify the waxy ratio is not known. That this gene is effective in producing a marked deficiency of waxy seeds has been established by BRINK (1925) and BRINK and BURNHAM (1927). They have shown that only when the $s_u W_z$ and $s_u w_z$ pollen classes arise from sugary homozygotes is the difference very marked. BRINK and BURNHAM (1927) suggest that the differential action occurs during the initial stages of pollen tube development. The retarded development of the $s_u w_z$ gametophyte may be reasonably accounted for, according to the authors, on the supposition that sugary sporophytes of the composition $s_u s_u W_z w_z$ pass on to the microspores a "modified" cytoplasm which is produced under the sustained action of the sugary gene. With this cytoplasm non-waxy and waxy nuclei interact

[1] This article is part of a thesis submitted to the Graduate School of the UNIVERSITY OF WISCONSIN in partial fulfillment of the requirements for the degree of Doctor of Philosophy, March, 1928.

Paper from the Department of Genetics, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN, No. 93. Published with the approval of the Director of the Station.

differentially. However, when pollen nuclei of the same constitution interact with "normal" cytoplasm passed on from $S_u s_u W_x w_x$ plants the capacities of the $s_u W_x$ and $s_u w_x$ gametophytes to form pollen tubes does not differ greatly, for no marked deficiency of waxy seeds in the sugary class as compared to that in the non-sugary class follows self-fertilization of such sporophytes.

The nature of the difference between the interaction of the non-waxy and waxy nuclei with the modified cytoplasm on the one hand and with normal cytoplasm on the other hand, may be sought, according to BRINK and BURNHAM (1927), in studies which give precise information on the effects of the S_u, s_u, and W_x, w_x, genes on the amount or character of the pollen reserves which are utilized during the initial period of pollen tube development. In their opinion, additional light may be thrown on the modifying effect of the sugary genes on the waxy ratio by studies of the effects these genes produce on the amount of endosperm reserves.

In 1926 the writer secured evidence relating to the effects of the S_u, s_u, and W_x, w_x, genes on endosperm fat metabolism. In this paper presentation is made of the results of comparative analyses of the amount and character of the crude fat reserves of $S_u W_x$, $S_u w_x$, $s_u w_x$ classes of endosperm borne on segregating ears, as related to the question of the curious modifying effect of the sugary gene on the waxy ratio. In this the precedent has been established by BRINK and BURNHAM (1927) who made a study of the weight of the above mentioned four classes of seed also borne on segregating ears. If it can be demonstrated that the effects of the factor difference W_x, w_x, on endosperm reserves are significantly different between the sugary and the non-sugary classes it may be surmised that the sugary gene has exerted a modifying action on the effects of the waxy gene. If, however, it can be demonstrated that the actions of the waxy and sugary genes in endosperm fat metabolism are independent of each other and that their effects are additive in the $s_u w_x$ type, it would seem that the above mentioned possibility would be a remote one. The evidence presented in this paper falls in line with the second hypothesis.

LITERATURE REVIEW

BRINK (1925) noted that the sugary gene in maize exerts a modifying action on the waxy ratio. It was shown that only 18.84 ± 0.26 percent of the waxy seeds resulted from self-pollinations of $s_u s_u W_x w_x$ plants, whereas 25 percent was the expectation on the Mendelian basis. In comparison, self-pollinations of closely related $S_u s_u W_x w_x$ plants yielded 24.82 ± 0.68 percent waxy seeds. The difference in percent of waxy seeds

between these types of self-pollinations was shown to be, without a doubt, statistically significant. It may be said that the deficiency in waxy seeds following self-pollination of $S_u s_u W_x w_x$ plants is of the order which characterizes that from self-pollinations of $S_u S_u W_x w_x$ plants or from crosses in which the pollen parent is heterozygous for waxy. That such deficiencies of waxy seeds are significant was established by BRINK (1925) who considered the direct cause to be a lower rate of growth of waxy pollen tubes. In this case the action of the W_x, w_x, genes on rate of pollen tube growth was thought to be directly expressed in the respective male gametophytes. BRINK and BURNHAM (1927) substantiated the conclusion that the sugary gene modifies the waxy ratio with further data bearing on the relative deficiencies of waxy seeds following self-pollinations of sugary and non-sugary plants, heterozygous for waxy, and selected from sister progenies. In addition two other important points were established. First the composition of the pistillate parent with respect to the sugary gene was shown to be without influence on the waxy ratio. This was taken to signify that the sugary gene exerts its disturbing influence through some action on the pollen only. Second, self-pollinations of $S_u s_u W_x w_x$ plants showed no significant difference in the proportion of waxy seeds in the sugary and non-sugary classes. According to the authors this demonstrates that the sugary gene does not exercise its initial differential effect directly in the mature pollen grain.

In considering what the mechanism might be by which the sugary gene operates to modify the waxy ratio the authors have centered attention on the amount of reserves in non-waxy and waxy pollen in sugary and non-sugary classes. These pollen types, from comparable plants, differed in chemical composition but this fact bore no evident relation to the question in hand. Information on the effect of the S_u, s_u, and W_x, w_x, genes on the amount of reserves produced was sought by a study of the respective weights of $S_u W_x$, $S_u w_x$, $s_u W_x$, and $s_u w_x$ seeds borne on segregating ears. It was found that the decrease in weight of waxy seeds as compared with non-waxy was not significantly different in the non-sugary and sugary seed classes.

The waxy character in maize is inherited as a simple recessive. The waxy endosperm starch colors purple in the presence of iodine in KI, while the non-waxy develops a deep blue color specific for unmodified starch. DEMEREC (1924) and BRINK and MACGILLIVRAY (1924) have shown that the effect of the waxy gene on modification of the starch reserve is manifest in pollen. BRINK and ABEGG (1926) in a study pertaining to the dynamics of the waxy gene sought information on the effects

produced by this gene on carbohydrate metabolism. ABEGG (1929) has shown that the waxy gene occasions marked changes in the fat metabolism of endosperm tissue only. The waxy endosperm tissue yields more ether soluble crude fat and its acid value is significantly greater than that from the non-waxy. These modifications are shown in much lower degree, if at all, in pollen, and seem to be absent in embryo tissue.

The sugary type of maize seed is primarily distinguished from that of the non-sugary by its wrinkled appearance, following drying. BRINK (1927) has found the sugary type to be approximately 12 percent lower in seed weight than non-sugary grain. In contrast to the plump starch grains of the non-sugary endosperm those of the sugary appear to be disrupted and are irregular in outline as if incompletely developed. Comparative chemical analyses of related non-sugary and sugary endosperm tissue made by LINDSTROM and GERHARDT (1926) show that the latter type is much higher in soluble carbohydrates and crude fat, but lower in starch. According to these authors the simple Mendelian pair of genes, S_u, s_u, exercise a great degree of control over the carbohydrate and fat metabolsim of maize endosperm which is not disturbed by hybridization. EMERSON (1924) found the sugary gene to be fully independent of the waxy gene in inheritance.

EXPERIMENTAL RESULTS

The analyses which follow have been made with the intention of characterizing the effects of the S_u, s_u, and W_x, w_x, genes on the amount and properties of endosperm crude fat. The four classes of endosperm tissue used for ether extraction and subsequent determinations of acid, saponification, and ester values came from a composite sample of seeds from five ears, each with a distribution of S_uW_x, S_uw_x, s_uW_x, and s_uw_x types approximating the expected proportions of 1:1:2:1. These ears[2] resulted from the backcross $s_us_uw_xw_x$ ♀ $\times S_us_uW_xw_x$ ♂ $+ 1/4s_uW_x$ ♂ made by BRINK and BURNHAM (1927) to test the hypothesis that the s_uW_x pollen type was influenced by the sugary gene in its rate of pollen tube growth to a certain extent. Only endosperm (plus pericarp) tissue was used for ether extraction, the embryos having been removed in the dry state with painstaking care. The method of preparation of these samples for extraction and the determination of the various fat values are identical with those employed in the comparative study of non-waxy and waxy maize grain as embodied in the paper by ABEGG (1929).

The four endosperms that are under consideration are genotypically

[2] The author is indebted to Doctor R. A. BRINK for the use of this material.

$S_uS_uS_uW_zW_zW_z$, $S_uS_uS_uw_zw_zw_z$, $s_us_uS_uW_zW_zW_z$, and $s_us_us_uw_zw_zw_z$. Any difference in crude fat content, let us say, between the first pair and likewise the second pair of types may be referred to the effect of the factor difference, W_z, w_z. When considering the mechanism by which the sugary gene modifies the waxy ratio it is desirable to know whether through some possible action of the sugary gene the effect of the waxy gene on amount of reserves has been modified. The above mentioned comparison of the differential effect of the W_z, w_z, genes in the non-sugary and sugary classes should afford evidence on this point. It is to be emphasized that since all four endosperm classes were borne on the same ear their development occurred in a uniform external environment, a condition necessary for critical information on the effect of particular genetic factors on reserves.

Comparative amounts of crude fat of non-waxy and waxy endosperm types in non-sugary and sugary classes

Consideration of the figures in table 1 shows that the difference of approximately 0.4 percent in crude fat yield, comparing non-waxy and waxy endosperm types, occurs in both the non-sugary and sugary classes. This indicates that the waxy gene operates to produce an increase in crude fat yield independently of the S_u, s_u, genes. In this sense the sugary gene shows no modifying effect on the actions of the waxy factor. The result considered here is of the same nature as that obtained by BRINK and BURNHAM (1927) who have shown that a difference of 1.35 percent in seed weight between the non-waxy and waxy types in the non-sugary class was approximated by a difference of 1.2 in the sugary class. The observed crude fat differences bear no evident relation to the question of the mechanism by which the sugary gene modifies the waxy ratio.

TABLE 1

Percentage of ether soluble crude fats extracted from four related types of maize endosperm.

	NON-SUGARY		SUGARY	
	Non-waxy	Waxy	Non-waxy	Waxy
Percentage* of crude fat	1.01 1.00 1.02	1.46 1.42 1.42	2.60 2.62 2.60	2.98 3.01 3.02
Average	1.01	1.43	2.61	3.00

* Based on dry weight of samples.

That maize seed weight and endosperm crude fat content are closely correlated seems probable. LINDSTROM and GERHARDT (1926) believe

that the lighter weight of sugary corn argues for their relatively high oil content. ABEGG (1929) has shown that a marked difference in crude fat content between non-waxy and waxy grain refers to their endosperm tissues. The same may hold true for the crude fat yield difference between non-sugary and sugary kernels. The results that BRINK and BURNHAM (1927) obtained on seed weight differences between the S_uW_x, S_uw_x, s_uW_x, and s_uw_x types would seem to be correlated with their endosperm crude fat content.

Comparative values of crude fat of non-waxy and waxy maize endosperm types in non-sugary and sugary classes

The saponification values given in table 2 represent single determinations. They are, therefore, only approximate estimations of the total fatty acids present in unit quantity of ether extract from the four endosperm types under consideration. A large quantity of endosperm tissue must be extracted with ether in order to obtain sufficient crude fat for hydrolysis with alcoholic potash. With the amounts of extract available it was not feasible to remove unsaponifiable substances such as phytosterols. These values may not, therefore, be representative of the true mean molecular weights of the fatty acids in the respective crude fats. Lack of sufficient extract was the greatest limiting factor in these experiments.

TABLE 2

Properties of ether soluble crude fats extracted from four related types of maize endosperm.

	NON-SUGARY		SUGARY	
	Non-waxy	Waxy	Non-waxy	Waxy
Saponification value	176	205	197	196
Acid value	76	117	84	97
Ester value	100	88	113	99

In each case the acid values recorded in table 2 represent averages of duplicate determinations, which agreed closely. They may be taken as fairly reliable relative measures of free fatty acids. The ester value represents the mgs. of KOH required to neutralize the combined fatty acids (neutral fat) present in unit quantity of the respective endosperm ether extracts. The ester value represents the difference between the saponification and acid values.

Difference in crude fat properties between these endosperm types do not seem to bear a definite relation to the question of the modifying action of the sugary gene on the waxy ratio. It may be seen from the

figures in table 2 that the difference between the non-waxy and waxy endosperm crude fats in saponification value in the non-sugary group is not apparent in the sugary group to a similar extent. Owing to lack of duplicate figures for such values no claim can be made that the particular value of the $s_u w_x$ crude fat indicates a modifying action of the sugary gene on the effect of the waxy factor. It is quite clear that the rise of approximately 40 in acid value of the $S_u w_x$ extract in comparison with that from $S_u W_x$ endosperm tissue is reduced to 13 in the sugary group. No doubt these figures are representative of the true acid values of such extracts. However, on calculation it proves that the acid value of the $s_u w_x$ type, or 97, is very close to expectation on the basis of the independent action of the waxy and sugary genes on fat metabolism. The ester values show a measure of regularity in difference between the non-waxy and waxy types in the non-sugary and sugary groups. Therefore no disturbing influence of the sugary gene on the activities of the waxy factor are apparent.

Additive effects of the waxy and sugary genes on fat metabolism in the $s_u w_x$ endosperm

Yield of crude fat

It would seem that the effects of the sugary and waxy genes on crude fat yield are additive and their action on processes which determine increases in ether soluble substances are independent of each other. Correlated with the W_x, w_x, factor difference is a difference of 0.4 percent crude fat and with the S_u, s_u, factor difference, one of 1.6 percent. Comparing $s_u w_x$ with $S_u W_x$ it is found that the difference of 2.0 percent between them is the sum of those determined individually for the factor differences, W_x, w_x, and S_u, s_u. According to the hypothesis of additive effects it is assumed that the ether soluble substances of the four endosperm types contain the various crude fat components in proportions as indicated in figure 1.

The $s_u w_x$ endosperm type also reveals the additive effects of the s_u and w_x genes on carbohydrate metabolism. The wrinkled appearance of the kernels and the red-purple color developed as a result of the iodine test for starch indicate that the genes under consideration have produced their modifying effects independently of each other in the $s_u w_x$ type. The nature of the connection between the crude fat yield and starch reserves remains unknown. It may be pointed out that in the waxy type an increase of 40 percent of ether extract is not associated with an ap-

preciable reduction in amount of starch as indicated by the analyses of BRINK and ABEGG (1926), but rather with a change in the properties of the starch, possibly one of degree of polymerization. In the sugary type, however, a percentage increase of 160 in ether extract is associated with a great reduction in amount of starch. It would seem that the latter starch does not vary in properties from ordinary starch.

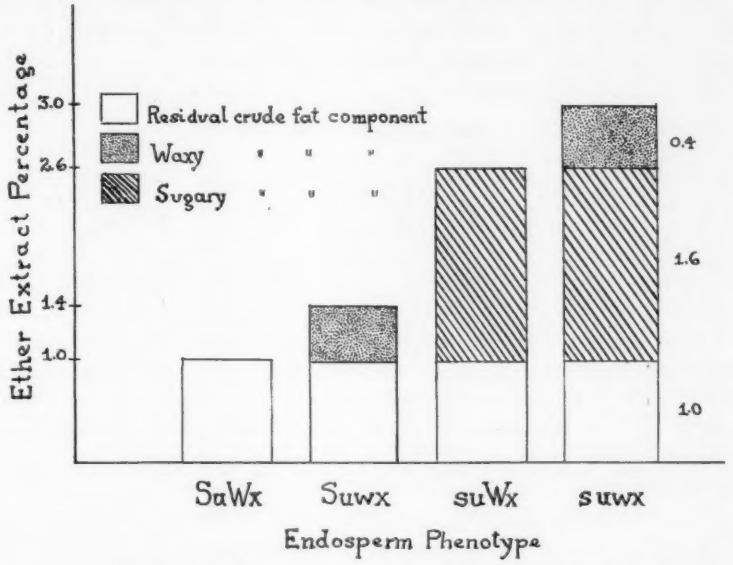

FIGURE 1.—The additive effects of the waxy and sugary genes in maize on reserves as shown by relative amounts of ether extracts from four endosperm types, borne on the same ear.

Since it is probable that crude fat content and seed weight are correlated to a significant extent it may be expected that the effects of the waxy and sugary genes on amount of reserves are additive. BRINK and BURNHAM (1927) have shown that the average seed weights of the S_uW_x, S_uw_x, s_uW_x, and s_uw_x classes borne on the same ears are respectively 175.25, 172.91, 155.16, and 153.05 milligrams. On a basis of the S_uW_x weight equalling 100 milligrams, it is found that a difference of -1.35 percent is attributable to the W_x, w_x, factor difference (in the non-sugary class) and one of -11.47 is associated with the S_u, s_u, factor difference. Their sum or -12.8 percent is in good agreement with the difference of -12.7 percent between the s_uw_x and S_uW_x seed weights. This would

seem to indicate that in the former type the activities of the waxy and sugary genes which relate to the production of reserves are independent of each other and consequently their effects are additive.

The figures recorded by PEARL and BARTLETT (1911) on crude fat content of yellow non-sugary, white non-sugary, yellow sugary, and white sugary F_2 classes of maize seed reveal the fact that the effects of

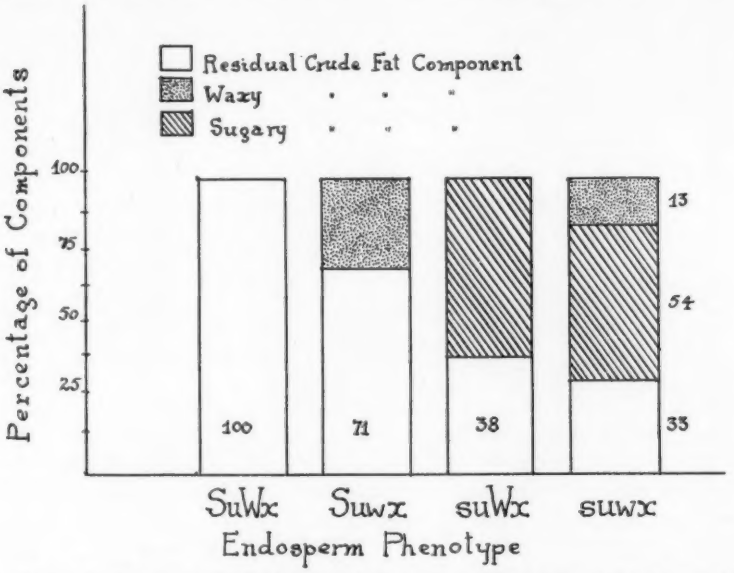

FIGURE 2.—Percentage distribution of crude fat components in ether extracts from maize endosperm, based on hypothesis of additive effects of waxy and sugary genes on fat metabolism.

the recessive genes, y and s_u, on fat metabolism are additive. The YS_u, yS_u, Ys_u, and ys_u seed classes have according to these authors corresponding crude fat contents of 5.03, 5.61, 8.51, and 9.09. A comparison of the yield of the first and second classes of seed gives a difference of 0.58 percent, associated with the factor difference, Y, y, (yellow versus white endosperm). Likewise with the S_u, s_u, factor difference there is associated one of 3.48 percent. The difference between the S_uY and s_uy classes is 4.06, exactly the sum of the individual differences determined respectively for the Y, y, and S_u, s_u, factor differences. It is therefore quite probable that in action on fat metabolism the white endosperm gene is independent of that of the sugary gene. It is interesting to find

that the effects of the Mendelian factor, *y*, independent in inheritance of the sugary factor as is the case with the waxy gene also, falls in line with the principle under discussion. As will be pointed out this fact may prove to be of future use.

Figure 2 shows graphically the percentage distributions of the various components in each of the four types of endosperm crude fat under consideration. In the next section, treating of the properties of these extracts, these percentage distributions will serve as a basis for the calculation of the effect produced on acid and ester value by each component in the $S_u w_z$, $s_u W_z$, and $s_u w_z$ crude fat.

Properties of crude fats

The following analysis of acid values recorded in table 2 is indicative of the probability that the waxy and sugary genes have produced their effects on properties of crude fats in the $s_u w_z$ endosperm independently of each other. The aim of this analysis is to demonstrate that the acid value of 97, characterizing the crude fat from the $s_u w_z$ endosperm, represents the additive effects of the waxy and sugary genes. The proportions of residual, waxy, and sugary crude fat present in 1 gram each of the ether extracts of the four endosperm types are shown in figure 2. These three components, varying as to presence and amount in each type of crude fat have particular corresponding acid values. For instance, 1 gram of the waxy extract ($S_u w_z$) is presumably composed of 71 percent of the residual crude fat and 29 percent of waxy crude fat, the latter component being related to the activities of the waxy gene. The term residual crude fat applies to the component characteristic of the $S_u W_z$ type and is presumably present in each of the other extract types in varying percentage. The acid value, based on one gram of this component, is 76. The acid value of 117 ($S_u w_z$) may be broken up into 54 and 63 corresponding to the 0.71 gram of residual crude fat and 0.29 gram of waxy crude fat respectively, which compose 1 gram of $S_u w_z$ ether extract. Likewise it is shown that the 0.62 gr. of the sugary crude fat component in 1 gram of the $s_u W_z$ extract has an acid value of 55. It may then be calculated that the corresponding acid values of three components in the $s_u w_z$ extract are 25, 48, and 28 respectively, giving a total calculated value of 101. This situation is depicted in figure 3. The calculated value closely approximates the actual value of 97. In the same manner it can be shown that the ester value of the $s_u w_z$ type is representative of the sum of the individual effects of the waxy and sugary genes on properties of crude fats. The calculated ester value is 106 as compared with an actual value of 99.

Since only one set of saponification numbers is available, better agreement would hardly be expected.

A further opportunity to test the hypothesis that the waxy and sugary genes act independently of each other in fat metabolism would seem to present itself in carrying through comparative analyses of amount and properties of crude fat of the endosperm classes derived from trihybrid backcrosses involving in addition to the W_x, w_x, and S_u, s_u, genes also the independent Mendelian factor pair Y, y, (endosperm color factors).

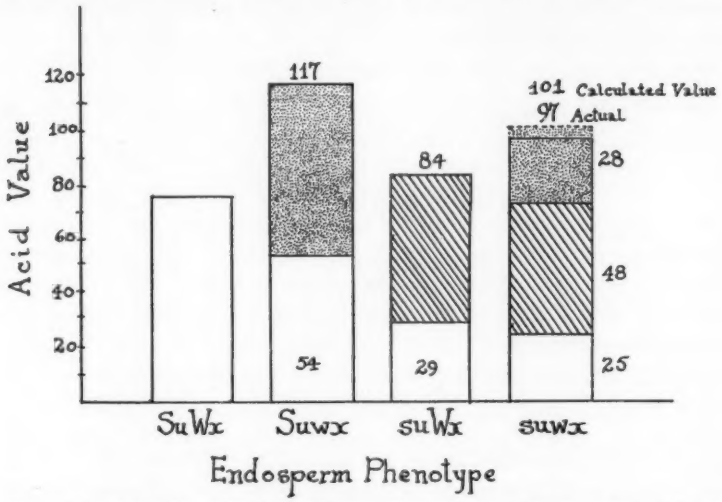

FIGURE 3.—Acid values of maize endosperm ether extracts. The component acid values were calculated on the basis of the hypothesis of additive effects of waxy and sugary genes on fat metabolism in $s_u w_x$ type. See figure 2 in text for legend.

Evidence exists which makes it probable that the y gene, operative in white endosperm tissue, produces an increase in amount of crude fat as well as in acid value. From a knowledge of the effects produced by this additional gene it would be possible to predict with considerable precision what the yield and properties of the crude fat from several of the endosperm classes in the trihybrid distribution should be. An all important condition that would have to be met in such an experiment is the use of the various classes of seed grown on the same ears in order to secure the benefit of a uniform effect of environmental factors on fat metabolism. Otherwise the effects of the particular genes involved might be overshadowed.

Additive effects of the waxy and sugary genes on fat metabolism in the $s_u w_x$ type of pollen

BRINK (1927) has shown that non-waxy and waxy pollen in the non-sugary class differed by 3.6 percent in yield of total crude fat (absolute alcohol and ether soluble substances combined) and by 3.2 percent in the sugary class. This result bears no evident relation to the mechanism by which the sugary gene operates to modify the waxy ratio. The data given by BRINK (1927) are amenable, however, to the following interpretation. The total crude fat content of $S_u W_x$, $S_u w_x$, $s_u W_x$, and $s_u w_x$ pollen would seem to indicate that the waxy and sugary genes are independent in their action on fat metabolism in the $s_u w_x$ male gametophyte and that their effects in $s_u w_x$ pollen are additive. The above mentioned types of pollen contain approximately 21.94, 25.54, 23.58, and 26.76 total crude fats, respectively. The sum of the individual percentage differences of $S_u w_x$ and $s_u W_x$, each in comparison with $S_u W_x$ pollen, is 5.24 (3.60+ 1.64). Comparing $s_u w_x$ with $S_u W_x$ pollen we find a difference of 4.32 percent. This is only in fair agreement with 5.24 percent but there is a suggestion in this result nevertheless which points to the additive effects of the waxy and sugary genes.

DISCUSSION

Data presented in this paper show that differences in amount and properties of ether soluble crude fat between the non-waxy and waxy endosperm types of maize in the non-sugary and sugary classes, respectively, bear no evident relation to the nature of the physiological actions whereby the sugary gene modifies the waxy ratio. The respective crude fat yields of $S_u W_x$, $S_u w_x$, $s_u W_x$, and $s_u w_x$ endosperm tissues, borne on the same ears, were found to be 1.0, 1.4, 2.6, and 3.00 percent. In this instance the sugary gene has not caused a disproportionate difference in yield of ether soluble substances between the non-waxy and waxy types. In both non-sugary and sugary classes a difference of 0.4 percent is associated with the W_x, w_x, factor difference. The indication is that the activities of the waxy and sugary genes on yield of crude fat are independent of each other, and it has been shown that their effects appear to be additive on $s_u w_x$ endosperm fat metabolism. This point of view is upheld by the fact that data on crude fat yields of $S_u Y$, $S_u y$, $s_u Y$, and $s_u y$ maize types recorded by PEARL and BARTLETT (1911) show clearly that the effects of the y and s_u genes on crude fat yield are additive in the $s_u y$ type. The above conclusion seems to apply likewise to the effects which the waxy and sugary genes produce on properties of endosperm crude fat.

Acid values of the S_uW_z, S_uw_z, s_uW_z, and s_uw_z endosperm crude fats proved to be 76, 117, 84, and 97 respectively. These figures might give rise to the belief that the differential effects of the W_zw_z factor pair were not equal in the non-sugary and sugary classes. However, this does not seem to be actually the case. In order to comprehend that the effects of the waxy and sugary genes are independent of each other and that their effects on acid values are additive in the s_uw_z endosperm it should be borne in mind that each unit of ether extract (amount on which acid values are based) of the above mentioned types probably contains varying percentages of different crude fat components, each having a particular acid value. On a basis of this assumption it is evident that 1 gram each of the non-waxy and waxy ether extracts differ decidedly in the non-sugary and sugary classes both in amount and in character of crude fat components present. Therefore the same differences in acid value between these types does not occur in both classes. Nevertheless, the differential effect of the W_z, w_z, genes is proportional to the variation in amount and kind of crude fat components existing between unit quantity of non-waxy and waxy ether extracts in non-sugary and sugary classes, respectively. This would seem to indicate that the waxy and sugary genes have produced their effects independently of each other. In the s_uw_z type the effects of these genes on acid values appear to be additive. There is no evidence for the belief that the sugary gene has acted differentially on fat metabolism of s_uW_z and s_uw_z endosperm types in any manner commensurable with its apparent differential action on s_uW_z and s_uw_z male gametophytes, which BRINK and BURNHAM (1927) hold to be in causal relationship with marked deficiencies of waxy seeds following self-pollinations of $s_us_uW_zw_z$ plants.

It has been pointed out that maize seed weight and endosperm crude fat yield seem to be correlated, a fact noted by LINDSTROM and GERHARDT (1926). A decrease of approximately 1.35 percent in weight of waxy seeds as compared with non-waxy is associated with an increase of 40 percent of endosperm crude fat in the former. BRINK (1927) found the sugary type to be 11.5 percent less in seed weight than the non-sugary type. The former exceeds the latter in endosperm crude fat content by 160 percent. It is suggested that the effects of the waxy and sugary genes on amount of reserves formed in s_uw_z maize seed are additive. This seems to be borne out by the fact that the s_uw_z type is differentiated from the S_uW_z seed class in seed weight by a percentage decrease of 12.7. The sum of the percentage decreases associated individually with the W_z, w_z, and the S_u, s_u, factor differences is 12.85 (1.35+11.5).

BRINK (1927) has shown that the total crude fat yield difference between non-waxy and waxy pollen is 3.6 percent in the non-sugary class and 3.2 percent in the sugary class. This indicates that the differential effect of the W_x, w_x, factor pair on total crude fat formed is independent of the effects of the S_u, s_u, genes. It is probable that the effects of the waxy and sugary genes on crude fat formation are additive in $s_u w_x$ pollen.

From present knowledge of the effects of these genes on reserves in pollen and endosperm it is not possible to predict what the mechanism is by which the sugary gene operates to modify the waxy ratio. Nevertheless, the hypothesis which BRINK and BURNHAM (1927) have advanced regarding the action of the sugary gene in producing a modified cytoplasm in sugary homozygotes centers attention on a new phase of the kinetics of the sugary gene in maize. So far, comparative analyses of carbohydrate and crude fat reserves of non-sugary and sugary endosperm tissues show that the sugary gene produces marked effects on carbohydrate and fat metabolisms. Chemical differences between non-sugary and sugary pollen have, moreover, been noted by BRINK (1927). The difference between these types of pollen in percentage of total crude fat and in the ether soluble component make it probable that the sugary gene modifies the fat metabolism of pollen. No evidence exists, however, which shows that such modifications are the results of the initial action of the sugary gene directly in pollen rather than those connected with its activities in the parent sporophyte. The hypothesis advanced by BRINK and BURNHAM (1927) on the probable differential action of the sugary gene on $s_u W_x$ and $s_u w_x$ gametophytes should stimulate interest in the question whether non-sugary and sugary sporophytes may be distinguished by physico-chemical differences. Evidence relating to this new phase of the kinetics of the sugary gene might afford some clue as to how this factor operates to modify the waxy ratio in maize.

SUMMARY

1. Information on effects of the waxy and sugary genes in maize on fat metabolism has been sought from data on comparative amounts and properties of ether soluble substances from $S_u W_x$, $S_u w_x$, $s_u W_x$, and $s_u w_x$ endosperm types, borne on the same ears.

2. The results of this study have been related to the mechanism by which the sugary gene operates to modify the waxy ratio in accordance with the hypothesis advanced by BRINK and BURNHAM (1927).

3. The crude fat yields of $S_u W_x$, $S_u w_x$, $s_u W_x$, and $s_u w_x$ endosperm

tissues, borne on the same ears, proved to be 1.0, 1.4, 2.6, and 3.0per cent respectively.

.4 The differential effect of the W_x, w_x, factor pair on endosperm crude fat yield appears to be equivalent in non-sugary and sugary groups. This conclusion agrees with that advanced by BRINK (1927) relative to such differential effects on amount of seed reserves and on total crude fat yield in pollen.

5. There is no evidence for the supposition that the sugary gene has acted differentially on fat metabolism of s_uW_x and s_uw_x endosperms in any manner commensurable with its apparent differential action on the s_uW_x and s_uw_x classes of male gametophytes.

6. Saponification, acid, and ester values of S_uW_x, S_uw_x, s_uW_x, and s_uw_x endosperm crude fats bear no evident relation to the nature of the physiological actions of the sugary gene in modifying the waxy ratio.

7. The data reported in this paper on crude fat yield and properties of ether soluble substances of the above mentioned endosperm types indicates that the activities of the waxy and the sugary genes on fat metabolism are independent of each other, and it had been shown that their effects are additive in s_uw_x endosperm tissue.

8. Relative differences in seed weights between S_uW_x, S_uw_x, s_uW_x, and s_uw_x types of maize, determined by BRINK (1927), would seem to indicate that the effects of the waxy and sugary genes on amount of reserves laid down are additive in the s_uw_x type. Likewise differences between corresponding pollen types in total crude fat content as determined by BRINK (1927) suggest that the effects of the waxy and sugary genes on fat metabolism in the gametophyte generation are additive in the s_uw_x pollen.

Grateful acknowledgment is made to Doctor R. A. BRINK for his supervision of this investigation and his suggestions in the preparation of this paper.

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INTERSPECIFIC HYBRIDIZATION IN NICOTIANA.

IX. FURTHER STUDIES OF THE CYTOLOGY OF THE BACKCROSS PROGENIES OF THE PANICULATA-RUSTICA HYBRID[1]

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Received August 11, 1928

INTRODUCTION

As a part of the program of study on interspecific hybridization in progress at the UNIVERSITY OF CALIFORNIA, the *paniculata-rustica* hybrid has been chosen for particular attention on account of certain unique features which it exhibits. Up to the present time it is the only interspecific Nicotiana hybrid from species differing in chromosome number which, although notably deficient in fertility, may yield progeny on selfing, and EAST (1921) has demonstrated that stable recombination products may be established from it. The problem of the manner in which these recombination products come into existence constitutes one of the problems of this study, but of even more interest is the question as to the relationship existing between the parental species.

In a preliminary report GOODSPEED, CLAUSEN and CHIPMAN (1926) demonstrated that the chromosome numbers of the parents were *rustica* = 24_{II} and *paniculata* = 12_{II}, and that the F_1 hybrid exhibited conjugation regularly according to the Drosera scheme, $12_{II}+12_I$. Cytological examination of plants in the backcross progenies demonstrated that the functional gametes of the F_1 hybrid were mostly of the category $12+i$, as respects chromosome number with i having an average value of approximately 6, as is to be expected from random distribution of univalents. In the *paniculata* backcross progeny however, a group of plants reported to have $12_{II}+12_I$ chromosomes constituted an exceptional class requiring further investigation. The studies reported in this paper demonstrate that this exceptional class occurs, but that instead of having $12_{II}+12_I$ chromosomes the number is approximately $12_{III}+12_I$.

These cytological studies disclosed the necessity for parallel studies of chromosome distribution and genetic phenomena as a proper means of elucidating behavior in the progeny of this hybrid. Accordingly a

[1] The author is very much indebted to Doctor R. E. CLAUSEN, under whose direction this investigation was carried on, especially for suggestions as to method and interpretation of data.

new series of studies has been projected in which it is hoped to make a reinquiry into the cytological phenomena of the F_1 hybrid and to study backcross and selfed progenies in successive generations with a view to determining the extent to which knowledge of the chromosomal situation may aid in an analysis of the genetic phenomena. The present paper, reporting more extended studies of the backcross progenies, contains information as to the chromosome number of the functional female gametes produced by the hybrid.

MATERIAL AND METHODS

Of the many available varieties of *rustica*, *pumila* (U.C.B.G.169/08) was chosen to represent the species, largely because it is a small, early-maturing variety more easily manipulated than the larger varieties. It is our smallest variety of *rustica* and under favorable conditions may mature in 10 to 12 weeks from sowing. The strain of *paniculata* (U.C.B.G. 10/07) used is one typical of this highly stable species. Descriptions and figures of these species are contained in SETCHELL'S (1912) account of the genus.

The F_1 hybrid exceeds both parents in vigor of growth and like other *paniculata-rustica* hybrids is partially fertile. When crossed back as a female parent to the parental species capsules are produced containing an average of approximately 40 seeds each. More difficulty is experienced in producing seed by self fertilization or by backcrossing as the male parent on the parental species and the number of seeds thus produced is always less per capsule.

The seed used in the present investigation was obtained in 1925 by hand pollination in the greenhouse of F_1 *pumila-paniculata* with *paniculata* and *pumila*. These seeds were sown in January 1928 and the plants were grown in six inch pots in the greenhouse. Chromosome numbers were determined by examination of aceto-carmine smears of PMC. In a few instances root-tip counts were made from material fixed in Navaschin's modified solution and stained with iron-haematoxylin. The drawings of chromosome garnitures were made with the aid of a camera lucida; a 2 mm. Leitz apochromatic oil immersion lens and ×8 aplanatic eyepieces were used in working with PMC and gave, at table level, figures magnified about 2700 diameters; a higher magnification was needed with root tips, so ×15 aplanatic eyepieces were used giving figures magnified about 5100 diameters.

F_1 paniculata-rustica × paniculata

In the *paniculata* backcross all of the seeds produced in five capsules were sown. These capsules contained a total of 206 seeds of which 141 germinated and 120 plants were raised to maturity. A few of the seedlings died in the cotyledon stage, and some plants died at various stages, principally from attacks of leaf miner and mosaic. Chromosome numbers of 83 of the plants which reached maturity were determined and are recorded in table 1, under the caption 1928. For comparison the previous

TABLE 1

Frequencies of chromosome numbers in progenies of F_1 rustica-paniculata ♀ × paniculata ♂

	CLASS 1, $12_{II}+i_I$													TOTAL	CLASS 2 CA. 48 CHROMOSOMES
Number of univalents	0	1	2	3	4	5	6	7	8	9	10	11	12		
1926	—	—	1	1	4	—	8	—	4	2	—	—	—	20	14
1928	—	5	2	3	13	16	16	6	6	2*	—	—	—	69	14
Total	—	5	3	4	17	16	24	6	10	4	—	—	—	89	28

* Including one plant with $13_{II}+8_I$ chromosomes.

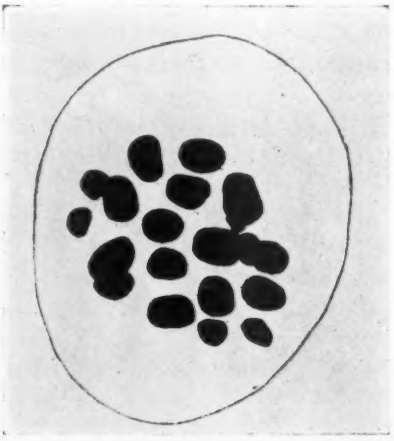


FIGURE 1.—$13_{II}+8_I$ derivative form F_1 *rustica-paniculata* ♀ × *paniculata* ♂ I-M, polar view, univalent chromosomes in gray. ×2700.

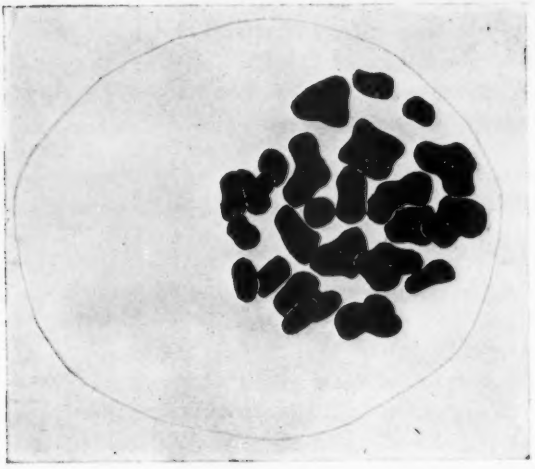
results of GOODSPEED, CLAUSEN, and CHIPMAN (1926) are also included in the table under the caption 1926.

An examination of the data contained in table 1 reveals the existence of two main classes as respects chromosome number: (1) 89 plants belonging to the series $12_{II}+i_I$; values of i ranging from 1 to 9 and (2) 28 plants having approximately $12_{II}+24_I$ or 48 chromosomes. The average value of i in class 1 was 5.2 as compared with an expected value of 6, based on purely random distribution. Included among these plants is an exceptional individual which uniformly exhibited $13_{II}+8_I$ in I-M. (figure 1). Not only was this plant unusual in having 13 bivalents but one of the univalents was distinctly smaller than the others. Equational non-disjunction in one of the univalents in the formation of the female gamete may possibly account for its production.

The 28 plants of class 2 require special comment. Accurate chromosome counts of these plants from PMC are impossible because of their variable behavior, but as a class they are easily recognized. Within a given acetecarmine mount the number of chromatin elements may vary from cell to cell from a minimum of about 24 to a maximum of approximately 36. A cell like that illustrated in figure 2 might possibly be interpreted as $12_{II}+12_I$, but closer examination showed that the larger chromatin elements were really not bivalents comparable to those shown in figure 1, but were more or less distinctly revealed, on focusing with the fine adjustment, as trivalents. The other extreme is represented in figure 3, depicting a cell from the same slide as the previous one. The range of variation in these cells, therefore, apparently lies between $12_{III}+12_I$ and $12_{II}+24_I$. Intermediate conditions are more usual, indicating either variation in the original formation of trivalents or successive stages in the separation of loosely attached univalents from the more closely associated bivalents.

In the previous paper of GOODSPEED, CLAUSEN, and CHIPMAN (1926), the 14 plants included in class 2 were recorded as $12_{II}+12_I$. There seems to be no doubt that this determination was incorrect, probably resulting from selection of cells approaching the condition illustrated in figure 2 of this paper, that is, $12_{III}+12_I$, and not $12_{II}+12_I$. As respects morphological features, these plants were identical with those assigned to class 2 in these studies.

Inasmuch, as accurate counts of chromosome number in plants of class 2 cannot be made by examination of aceto-carmine smears of PMC attempts were made to make counts from root-tip preparations. In making



FIGURES 2 and 3.—Class 2 derivative from F_1 *rustica-paniculata* ♀ × *paniculata* ♂. Figure 2: I-M, polar view showing apparently 25 chromatin elements (really $12_{II}+12_{I}\pm$). Figure 3: I-M, polar view from same anther showing other extreme condition, that is about 35 chromatin elements. ×2700.

FIGURE 4.—Somatic plate of a class 2 derivative from F_1 *rustica-paniculata* ♀ × *paniculata* ♂ showing 46 chromosomes. × 5100.

FIGURE 5.—Somatic plate of a derivative from F_1 *rustica-paniculata* ♀ × *paniculata* ♂ showing 80 chromosomes. × 5100.

these determinations advantage was taken of the fact that division figures in older roots exhibit shorter and thicker chromosomes than those of younger roots. The two plants determined had 46 chromosomes. A plate from one of them is illustrated in figure 4.

In addition to the above plants a single plant was found which exhibited a very high chromosome number. Attempts to determine its chromosome number from PMC were unsuccessful but root-tip counts gave 80 as its somatic number (figure 5).

As regards morphological features, the plants of this backcross progeny varied to such an extent that no two of them were identical, as was also reported for the previous studies of GOODSPEED, CLAUSEN, and CHIPMAN

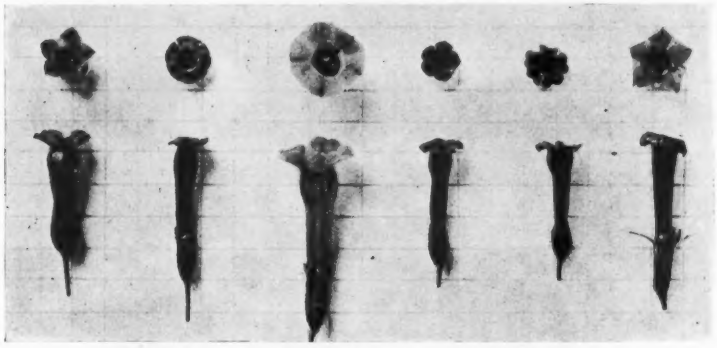


FIGURE 6.—Flowers from six different class 1 derivatives of F_1 *paniculata-rustica* ♀ × *paniculata* ♂. The scale in these figures is indicated by the background which is ruled in 5 mm. squares.

(1926). This is no doubt to be expected for individuals belonging to class 1, but those belonging to class 2 appeared to be no less variable, as may be seen by comparison of figures 6 and 7. In view of the fact that these plants arise through union of a somatic gamete produced by the F_1 hybrid with a normal *paniculata* gamete they should be identical in constitution and in morphological features. Their diversity evidently arises from the fact that the production of somatic gametes by the F_1 hybrid is usually accompanied by irregularities in chromosomal distribution, particularly by loss of a few chromosomes, so that the "somatic" gametes do not possess the entire somatic complement of chromosomes as is shown by the root-tip counts reported above. Preliminary observations on fertility failed to disclose any obvious relations with chromosome number. Fertility was uniformly low in class 2, to such an extent that no seed was obtained

from selfing or backcrossing to *paniculata*, but strangely enough some seed was secured from the backcross to *rustica*. In class 1 fertility was more variable and a limited amount of seed was secured by backcrossing to *paniculata*. On the whole fertility in this group of plants appeared to be lower than that of the F_1.

The above results demonstrate that the viable female gametes of F_1 *paniculata-rustica*, as shown by the chromosome counts of *paniculata* backcross progenies, are apparently produced in two distinct ways: (1) through normal separation of bivalents and approximately random distribution of the univalents and (2) through equational division of the entire chromosomal complement, accompanied by minor irregularities, resulting

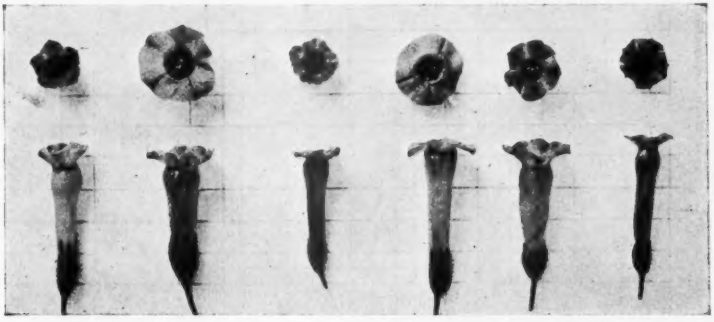


FIGURE 7.—Flowers of six different class 2 derivatives of F_1 *paniculata-rustica* ♀ × *paniculata* ♂.

in the production of "somatic" gametes having approximately the full somatic complement of chromosomes of the F_1 hybrid. Rarely other types of gamete formation may occur, as is evidenced by the production of an 80 chromosome plant in this progeny which probably arose through the production of an F_1 gamete having approximately double the somatic complement of chromosomes.

F_1 paniculata-rustica × rustica

In the rustica backcross all of the seeds produced in five capsules were sown. These capsules contained a total of 183 seeds of which 94 germinated and 76 plants were raised to maturity. As in the previous population some seedlings died in the cotyledon stage and some of the plants failed to reach maturity principally because of insect and mosaic attack. Chromosome numbers of 67 plants were determined and are recorded in table 2 under the caption 1928. The previous results of GOODSPEED,

TABLE 2

Frequencies of chromosome numbers in progenies of F_1 rustica-paniculata ♀ × rustica ♂,

	CLASS 1, $h_{II}+k_I$ ($h+k=24$)													TOTAL	CLASS 2 CA 60 CHROMOSOMES
Number of bivalents + univalents	12_{II} + 12_I	13_{II} + 11_I	14_{II} + 10_I	15_{II} + 9_I	16_{II} + 8_I	17_{II} + 7_I	18_{II} + 6_I	19_{II} + 5_I	20_{II} + 4_I	21_{II} + 3_I	22_{II} + 2_I	23_{II} + 1_I	24_{II} + 0_I		
1926	—	—	—	—	—	—	7	—	4	—	—	—	—	11	
1928	—	—	—	3	8*	12	5	—	3	1	—	—	—	32	35
Total	—	—	—	3	8	12	12	—	7	1	—	—	—	43	35

* Including one plant with $16_{II}+9_I$ chromosomes.

CLAUSEN, and CHIPMAN (1926) are also included in the table under the caption 1926.

An examination of the data contained in table 2 again reveals the existence of two main classes as regards chromosome number (1) 43 plants belonging to the series $h_{II} + k_I$ with values of h ranging from 15 to 21 and k from 9 to 3, and (2) 35 plants having approximately $24_{II} + 12_I$ or 60 chromosomes. The average value of k in class 1 was 6.46 or to make the situation directly comparable with the data from the *paniculata* backcross, an average of 5.54 univalents are distributed to the *rustica* backcross

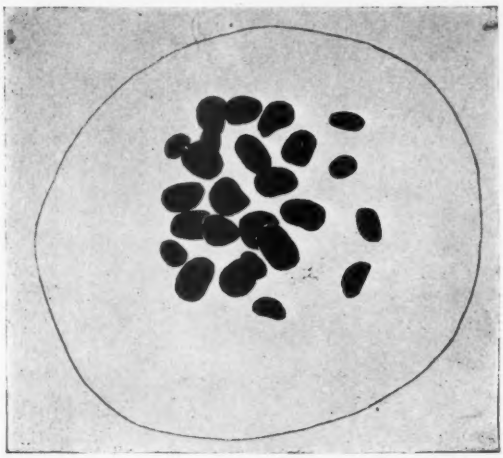
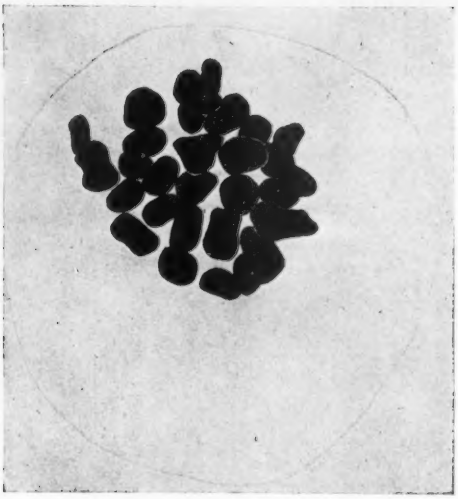
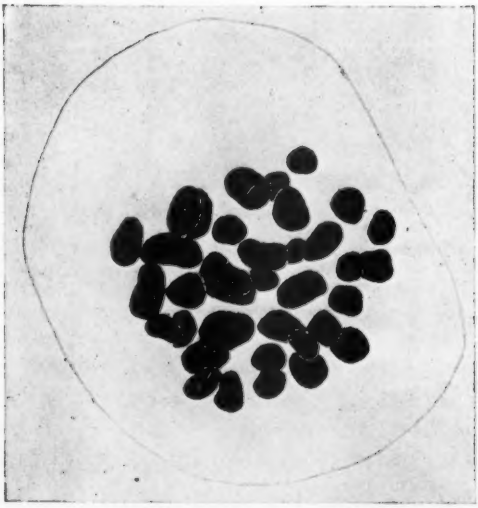


FIGURE 8.—$16_{II}+9_I$ derivative from F_1 *rustica-paniculata* ♀ × *rustica* ♂ I-M, polar view, univalent chromosome in gray. ×2700.

progeny by the F_1 hybrid parent. Included among these plants is an exceptional individual which uniformly exhibited $16_{II} + 9_I$ in I-M (figure 8). The bivalents were all clearly such and could not be confused with univalents. This plant is comparable with the $13_{II} + 8_I$ individual in the *paniculata* derivatives and probably also arose by equational non-disjunction in one of the univalents in the formation of the female gamete.

The 35 plants of class 2 are comparable with the class 2 plants of the *paniculata* backcross and arose in the same manner. Accurate chromosome counts of these plants from PMC are impossible because of their variable behavior, but as a class they are easily recognized. Within a





FIGURES 9 and 10.—Class 2 derivative from F_1 *rustica-paniculata* ♀ × *rustica* ♂. Figure 9: I-M, polar view showing apparently 28 chromatin elements. Figure 10: I-M, polar view, from same anther showing other extreme condition, that is, 35 chromatin elements ($24_{II}+12_{I}\pm$). ×2700.

given aceto-carmine mount the number of chromatin elements may vary from cell to cell from a minimum of about 24 to a maximum of approximately 36. A plate with apparently $24_{II} + 5_{I}$ is shown in figure 9. Rarely, plates may be found having apparently 24_{II} but in reality such figures are to be interpreted as $12_{III} + 12_{II}$ the trivalent nature of some of the chromatin elements being more or less distinctly revealed on focusing with the fine adjustment. Other PMC of the same anther may exhibit approximately 36 chromatin elements ($= 24_{II} + 12_{I}$) as shown in figure 10. Intermediate conditions are more usual, indicating either variation in the

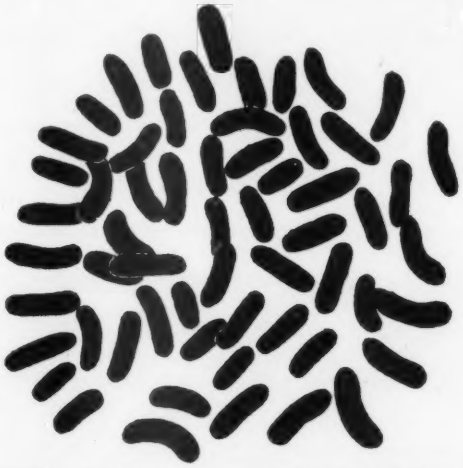


FIGURE 11.—Somatic plate of a class 2 derivative from F_1 *rustica* ♀ × *rustica* ♂ showing 59 chromosomes. ×5100

original formation of trivalents or successive stages in the separation of loosely attached univalents from the more closely associated bivalents. Inasmuch as accurate counts of chromosome number in plants of class 2 cannot be made by examination of aceto-carmine smears of PMC attempts were made to make counts from root-tip preparations. A characteristic plate from one of these roots, exhibiting 59 chromosomes, is shown in figure 11. Determination of four other plants gave 58, 60, 58, and 56-58 (?) chromosomes.

As regards morphological features the plants of this backcross progeny were much less variable than the *paniculata* derivatives, although the diversity was so great that no two plants were identical. All were pre-

vailingly *rustica* in type and many closely resembled the F_1 hybrid in general features. Variability is obviously to be expected among individuals belonging to class 1; but those belonging to class 2 were also variable, although not so strikingly so as the class 2 plants of the *paniculata* back-

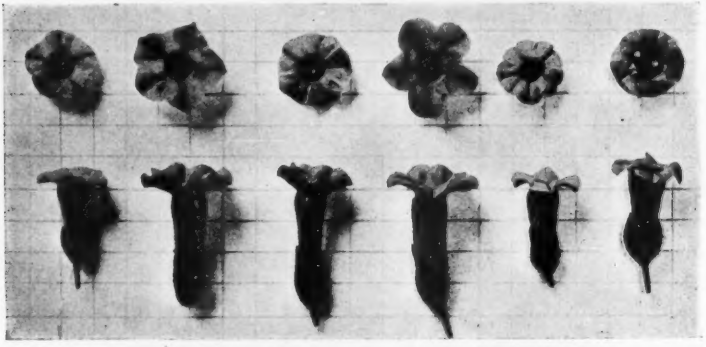


FIGURE 12.—Flowers of six different class 1 derivatives of F_1 *paniculata-rustica* ♀ × *rustica* ♂.

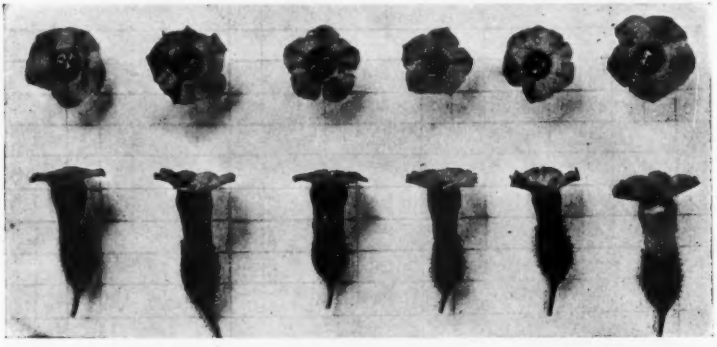


FIGURE 13.—Flowers of six different class 2 derivatives of F_1 *paniculata-rustica* ♀ × *rustica* ♂.

cross. Characteristic flower types are illustrated in figures 12 and 13. The diversity among these class 2 plants evidently arises from the fact that the production of somatic gametes by the F_1 hybrid is usually accompanied by irregularities in chromosome distribution, particularly by the loss of a few chromosomes, so that the "somatic" gametes do not possess the entire somatic complement as is shown by the root-tip counts

reported above. Apparently, however, the absence of one or two univalents in a $24_{II}+12_{I}$ plant does not cause such a distinct morphological change as a corresponding absence in a $12_{II}+24_{I}$ plant. Preliminary observations on fertility failed to disclose any obvious relation with chromosome number. Fertility was usually high in plants of class 1 as measured by seed production on selfing and backcrossing reciprocally to *rustica*, but at least one plant tested was completely sterile. Fertility in class 2 plants was even higher, only one plant failing to give selfed seed, and all were reciprocally backcross fertile. It was also possible to get seed from reciprocal backcrosses of class 2 plants to *paniculata*.

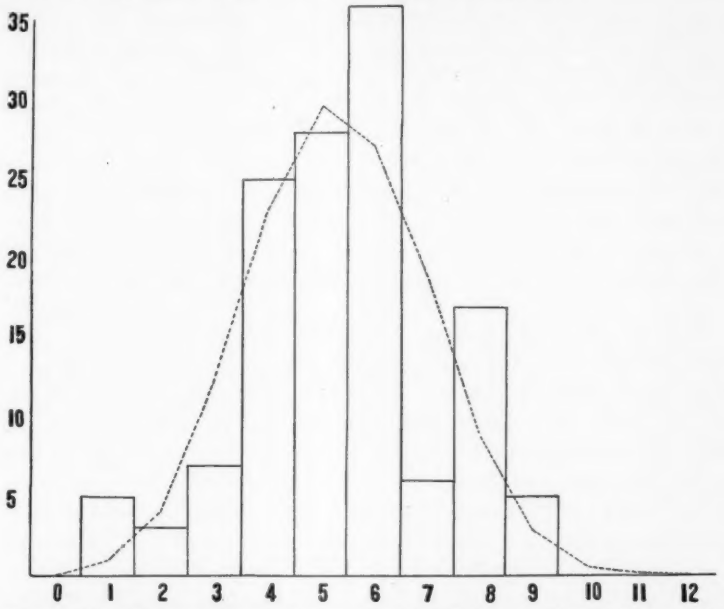


FIGURE 14.—Frequency polygon showing actual distribution of values of i from table 3 compared with calculated values obtained by expansion of binomial 132 (.552+.448).[12]

The above results demonstrate that the rustica backcross progeny arise from the same two classes of gametes as the *paniculata* backcross progeny; namely, (1) those produced by normal separation of bivalents and approximately random distribution of univalents and (2) somatic gametes from equational division of the entire chromosome complement accompanied by minor irregularities.

TABLE 3

Distribution of values of i in the production of viable gametes of class 1 by F_1 paniculata-rustica ♀.

i =	0	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL
paniculata backcross	—	5	3	4	17	16	24	6	10	4	—	—	—	89
rustica backcross	—	—	—	3	8	12	12	—	7	1	—	—	—	43
Total observed	—	5	3	7	25	28	36	6	17	5	—	—	—	132
Calculated 132 $(.555+.445)^{12}$	*.1*	*1.1*	*4.7*	*12.7*	*23.1*	*29.5*	*27.1*	*19.2*	*9.1*	*3.5*	*.85*	*.15*	*.007*	*131.1*

DISCUSSION

Inasmuch as the chromosome counts of the backcross progenies are in agreement as to the character of the viable female gametes produced by F_1 *paniculata-rustica*, they will be considered together in this brief discussion. As respects the regular gametes; that is, those resulting from normal disjunction of the bivalents and approximately random distribution of the univalents, the combined data of table 3 show that the average number of univalents included in these gametes is $5.38 \pm .11$. These distributions exhibit little evidence of selective viability of any chromosome number class, aside perhaps from the five gametes containing only one univalent in the *paniculata* backcross series. With an average of 5.38 univalents per gamete, the chance of inclusion of each univalent becomes $5.38 \div 12 = 0.448$, and a comparable calculated distribution may be obtained by expansion of the binomial expression, $132(.552+.448)^{12}$ As shown by the frequencies in table 3 and graphic representation in figure 14, the calculated and observed distributions are in close agreement. Apparently, therefore, the slight deviation from purely random distribution exhibited in these results is to be ascribed to chance elimination of univalents. Direct studies of "tetrads" are in harmony with this conclusion, for they exhibit a small percentage of microcytes and micronuclei.

The production of "somatic" gametes is also attended by slight irregularities in chromosomal distribution, as is shown by the variations in morphological features of plants arising from them and also by direct determination of chromosomes numbers of such plants. Preliminary observations of F_1 PMC indicate that dyad production is very rare, but some of the instances which have been observed exhibit micronuclei and microcytes in addition to the two primary cells. Apparently this type of gamete production is more frequent in EMC than in PMC. The evidence of irregularity is in harmony with observations on dyad formation in other hybrids, as for example F_1 *sylvestris-tomentosa* (GOODSPEED and CLAUSEN, 1928), a high percentage of the dyads of which exhibit micronuclei and microcytes. In the *tabacum* haplonts also these authors report similar irregularities in dyad production. It is possible that in many instances such imperfect dyads, particularly those representing minus deviations in chromosome number, are non-functional, which may account for the observation of BLAKESLEE, MORRISON and AVERY (1927) that the offspring of haploid Daturas are mainly diploid, and that such chromosomal variations as occur are in the plus direction. KARPECHENKO'S (1927) determinations of chromosome numbers in the progeny of F_1 Brassica-Raphanus hybrids likewise indicate irregularities in the pro-

duction of somatic gametes, but they also appeared uniformly to be in a plus direction. No doubt minus deviations are to be expected more frequently than plus ones, and in the material here reported only such irregularities were actually demonstrated, but this may be due merely to the small number of accurate determinations which were made.

It has usually been assumed that lack of conjugation promotes the production of somatic gametes. FEDERLEY (1928) and ROSENBERG (1928) in recent summaries bearing on this problem go so far as to state that in the cases of somatic gamete formation so far observed there is either complete or partial failure of pairing. Such a causal relationship between lack of pairing and somatic gamete formation may be present in most cases; but in the present instance observations of PMC indicate a highly regular conjugation according to the Drosera scheme, despite the fact that 32 percent of the viable female gametes are of the somatic category.

Sterility of interspecific hybrids is usually assumed to depend upon selective elimination of certain classes of gametes and in some instances there is objective evidence for this assumption. Thus it was shown by GOODSPEED and CLAUSEN (1927) that the predominating classes of viable gametes of the F_1 *sylvestris-tabacum* hybrid were those in which only 0-3 univalents were included. Here F_1 sterility may be explained in part as the result of inviability of most gametes having from 3-11 singles, and since according to chance distribution most gametes would be in this category, the sterility exhibited would be correspondingly high. However, in the case of F_1 *paniculata-rustica* this explanation does not apply inasmuch as the viable gametes apparently represent a random sample as respects chromosome number of the entire series. It is still possible that the sterility is selective, although the cytological data supply no positive evidence as to the manner in which the elimination of gametes is effected.

The bearing of these results upon the genetic findings hitherto reported, especially those of EAST (1921), is not entirely clear. It is impossible to bring cytological findings into proper relation with genetic results unless chromosomal determinations are made in connection with the genetic experiments. It would seem reasonable, however, to assume that the production of such a high proportion of somatic gametes must have a significant effect upon the character of the F_2 progeny, and it may also be possible that they play an important part in the production of constant *rustica* derivatives differing from the original parental type. These, however, are matters which we hope to take up in future studies in this series.

SUMMARY

1. Considering the two backcross progenies together, results of individual chromosome counts of 195 plants indicate that 68 percent of the viable female gametes of F_1 *paniculata-rustica* arise through normal distribution of bivalents accompanied by approximately random distribution of univalents.

2. The remaining 32 percent of viable gametes contain approximately the total somatic chromosome number of the F_1 hybrid.

3. In the first type of distribution, the average number of univalents included in the viable gametes was 5.38 as compared with an expectation of 6, based on purely random distribution. The evidence indicated that this slight deviation from expectation is to be ascribed to elimination of chromosomes during meiosis rather than to selective viability of different classes of gametes.

4. Root-tip counts of plants arising from somatic gametes demonstrate that these gametes rarely contain the complete somatic complement of chromosomes; but that in their production a few chromosomes are usually eliminated. The morphological diversity of the plants arising from these gametes is in agreement with this demonstration.

5. Occasional deviations from the above described methods of gamete formation were also found, specifically evidence of equational nondisjunction in the distribution of univalents and of doubling of the chromosome number in the production of somatic gametes.

6. Sterility of the F_1 is evidently not primarily due to inviability of gametes resulting from random distribution of univalents, inasmuch as viable gametes apparently represent a random sample, as respects the chromosome number, of the entire gametic series.

7. While the application of these results to the genetic phenomena is not self-evident, there can be no question as to the necessity of determining cytological conditions step-by-step in order to arrive at a proper analysis of the breeding data.

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INTERSPECIFIC HYBRIDS IN CREPIS. III. CONSTANT FERTILE FORMS CONTAINING CHROMOSOMES DERIVED FROM TWO SPECIES

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Received August 12, 1928

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INTRODUCTION

In a preliminary report upon interspecific hybrids in Crepis (COLLINS and MANN, 1923) attention was called to the possibility of securing constant fertile forms possessing a chromosome complex derived from two distinct species. The cross, *C. biennis* L. (n=20)×*C. setosa* (n=4) Hall. f. has produced a number of fertile races differing from each other in phenotype and in number of chromosomes. It is the purpose of this paper to describe the first of these constant forms to be secured and to discuss the evolutionary significance of such races.

CONJUGATION OF BIENNIS CHROMOSOMES

Crepis biennis, a species with 20 pairs of chromosomes, has been crossed successfully with two other species of Crepis, namely *C. parviflora* Desf. and *C. rubra* L. During gamete formation in these F_1 hybrids Mrs. LESLEY found that as in the *biennis-setosa* F_1 the 20 *biennis* chromosomes conjugate to form 10 pairs while the chromosomes from the other species remained as univalents and were distributed at random.[1] We have also observed this phenomenon in a more recently made hybrid of *biennis* and *setosa*.

Each gamete of the F_1, therefore, receives 10 *biennis* chromosomes

[1] Data from Doctor MARGARET MANN LESLEY, formerly instructor in genetics.

together with a random number from the haploid set of the other species. The regular conjugation of the *biennis* chromosomes to form 10 pairs in F_1 prevents the recovery of the parent species in subsequent generations as is possible in the majority of partially fertile species hybrids, and at the same time sets up a mechanism for the production of constant forms having 10 pairs of *biennis* chromosomes with or without chromosomes from the other species. As reported in the preliminary account, these F_1 hybrids when backcrossed with *biennis* produce plants having 30 *biennis* chromosomes which form 15 pairs during gametogenesis. Additional evidence is now available (detailed later in this paper) which indicates clearly that the 10 *biennis* chromosomes in the F_1 gametes may conjugate to form 5 pairs. There are, therefore, in the gametes of *biennis* four sets of chromosomes each containing five members which can form bivalents under appropriate conditions. We now have additional evidence to support the view that *bennis* is an octoploid species.

ORIGIN OF CREPIS ARTIFICIALIS (N=12), A CONSTANT HYBRID FORM FROM BIENNIS (N=20)×SETOSA (N=4)

The F_1 plants from the cross *biennis*×*setosa* were similar in phenotype to *biennis*. Plants of the F_2 and subsequent segregating generations exhibited great diversity in phenotype and fertility including *biennis*-like plants, plants showing *setosa*-like types on an enlarged scale, and plants with some *setosa* characters superimposed upon *biennis* characters.

The formation of viable seeds in a number of Crepis species appears to be influenced by environmental conditions at the time of pollination. A given plant may set seed well at one time and at another with changed conditions (cool, cloudy weather) appear sterile or almost so. It has, therefore, been impossible to determine accurately the degree of fertility of hybrid plants. However, those with more pronounced *setosa*-like characters were regularly less fertile than plants which more closely resembled *biennis*. Due to this situation the subsequent generations consisted largely of *biennis*-like plants. Occasional fertile, or partially fertile plants having some *setosa* characters intermingled with *biennis* characters have appeared. One such F_4 plant ($26.47P_2$) obviously had formed a working combination of chromosomes from both *biennis* and *setosa*. This plant was the beginning of the new form which is referred to here as *Crepis artificialis* for convenience in reference and because it represents a distinct new type. (The taxonomic treatment of this form will be published later).

Chromosomes of *C. artificialis*

The somatic chromosome counts reported here were obtained from root tips fixed in chromacetic formalin as used by BELLING (1928) imbedded in paraffin and sectioned in the usual manner. The stain used was Haidenhain's iron-haemotoxylin. The meiotic counts are from pollen mother cells. Buds of the right size were fixed in Carnoy from 2 to 12 hours, washed in absolute alcohol and run through 95 percent and 80 percent into 70 percent alcohol in which they were kept until examination. To make a preparation from this material the florets were dissected out of a bud and broken up on a slide in a drop of acetocarmine. The large lumps of debris were removed, and a cover glass was put on and sealed with a gum mastic paraffin mixture. Meiotic figures obtained in this way were generally superior to those made from fresh material. Usually more pollen mother cells were to be found, the contrast between chromosomes and cytoplasm was better, and the spindle fibers could be seen.

A count of root-tip chromosomes of this original *artificialis* plant ($26.47P_2$) was not made, but scanty PMC material indicated from 10 to 15 pairs. Study of progeny, however, strongly indicated that it contained 12 pairs. Thus when crossed with *setosa* two plants were secured each having 16 chromosomes. Four of these chromosomes came from *setosa*, the remaining 12 from 26.47 P_2.

Culture 26.040 (table 1) includes the selfed progeny from this original plant, and three other cultures, 27.107, 27.114 and 27.116, contain selfed

TABLE I

Number of chromosomes in plants of C. artificialis

CULTURE NUMBER	CHROMOSOME NUMBER					TOTAL PLANTS EXAMINED
	24	25	26	27	36	
26.040	24	2	—	1	1	28
27.107	6	—	—	—	—	6
27.114	2	1	1	—	—	4
27.116	4	2	1	—	—	7
TOTALS	36	5	2	1	1	45

progeny from three different 24 chromosome plants of the 26.040 culture. Occasional irregular pairing or absence of conjugation of one or two pairs of chromosomes has been observed during meiosis in *artificialis* plants which we believe accounts for the extra chromosome plants listed in table 1.

The reason for the irregular pairing of some chromosomes is obscure but it appears to be a condition characteristic of some *biennis* chromosomes. Preliminary studies on this species indicated 40 as the diploid number. ROSENBERG (1918) also first reported 40 for *biennis* and later (1920) found 42 chromosomes. MARCHAL (1920), however, reported only 16 pairs in PMC. Recently we have found *biennis* plants having 39

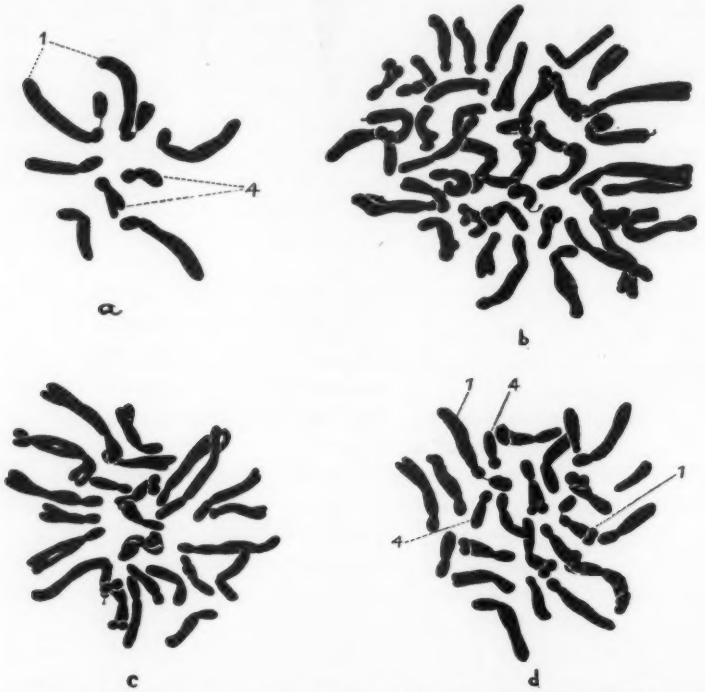

FIGURE 1.—Chromosomes from root tip cells of (a) *Crepis setosa* 27.1036P_4; (b) *C. biennis* 27.123P_2 showing 41 chromosomes; (c) F_1 hybrid *C. setosa*×*C. biennis* 27.008 P_1; (d) *C. artificialis* 26.040 P_{44}; the pairs from *setosa* are marked as in (a). Magnification ×2850.

and 41 chromosomes respectively and M. NAVASHIN has found plants having 42 and 45 chromosomes, (unpublished data). We see in these results a very probable explanation for the difference in chromosome number as found by different investigators. This pecularity of *biennis* is carried over into *artificialis* causing the occasional appearance of extra chromosome plants as shown in table 1.

The pollen was examined from 46 plants. There were two classes in regard to ability to take acetocarmine stain, one class staining deeply and readily, the other showing very little or no stain. There was approximately 57 percent of poorly stained pollen and 43 percent deeply stained pollen. The latter was considered as good pollen. The good pollen showed some variation in size but the majority of it was of medium size. The amount of good pollen on different plants varied from 15 to 70 percent, with most plants showing 45–50 percent.

The chromosome complex of *artificialis* (figure 1d) consists of 10 pairs from *biennis* and two pairs from *setosa* as evidenced by the following considerations: (1) The regular pairing of the *biennis* chromosomes to form 10 pairs insures the continuation of this number in subsequent progenies; (2) the *setosa* characters of *artificialis* indicated the presence of *setosa* chromosomes, and (3) the F_1 hybrids of *artificialis* with *setosa* exhibit a 7 paired and 2 unpaired type of conjugation. This latter is a consequence of the conjugation of the 10 *biennis* chromosomes to form 5 pairs and of the 6 *setosa* chromosomes to form 2 pairs and 2 singles according to the scheme shown in the following diagram in which *B* represents chromosomes from *biennis* and *S* chromosomes from *setosa*.

gamete of *artificialis* = 12 chromosomes $(10B+2S)$

$\times$ = 16 chromosomes in $F_1 \left\{ \begin{matrix} 10B(5_{II}) \\ 6S(2_{II}+2_I) \end{matrix} \right\} 7_{II}+2_I$

gamete of *setosa* = 4 chromosomes $(4S)$

In *setosa* the four different chromosomes are easily distinguishable, figure 1a. One long chromosome has a subterminal constriction to which a large satellite is attached by a thin thread. A second about equally long has the subterminal constriction but lacks the satellite. The third is similar to the second but is shorter, while the fourth is very small with a subterminal constriction.

The two *setosa* chromosomes present in the *artificialis* complex are quite readily recognized by their morphological characters and each is present twice. These are the first and fourth types mentioned above. This being the case the univalents observed in PMC's of the F_1 *artificialis* $\times$ *setosa* should exhibit two different size types and this they do even in the PMC's where individuality is less marked than in root tip cells. See figure 2.

MORPHOLOGY OF C. ARTIFICIALIS

Crepis artificialis is an annual, first forming a rosette from which the flowering stalk is produced. The rosette leaves are pinnately divided with large lyrate terminal lobe, see figure 3. They show the developmental variation common to this type of plant and also perhaps some genic heterogeneity exists. The leaves are more petiolate than are *biennis* leaves and are covered with a fine pubescence.

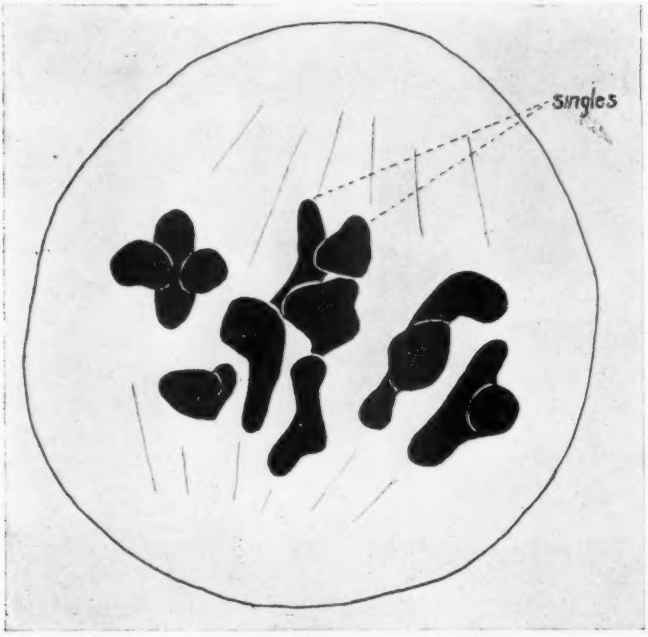

FIGURE 2.—A meiotic metaphase in a pollen mother cell of an F_1 plant, *C. artificialis*×*C. setosa*, (27.86 P_1) showing 7 pairs and 2 singles. Magnification, × 3700.

The stems which are sparsely hairy with *setosa*-like hairs are from 30 to 60 cm. tall depending upon growing conditions. The buds bear long, non-glandular hairs on the bracts. The flower heads are from 25–38 m.m. in diameter when open, which is much larger than *setosa* flowers and about the size of *biennis* flowers. The stigmas are dark colored like those of *setosa* and the achenes while, large like *biennis*, are not beaked. A comparison of the characters of *artificialis* with those of its parent

species shows *artificialis* to be *biennis*-like in about seven characters and *setosa*-like in about eight and to be intermediate or compound in some four other characters. See table 2.

A TRIPLOID PLANT OF C. ARTIFICIALIS

One of the extra chromosome plants listed in table 1 is a triploid *artificialis*, having 36 chromosomes. This plant ($26.040P_{29}$) was slightly more

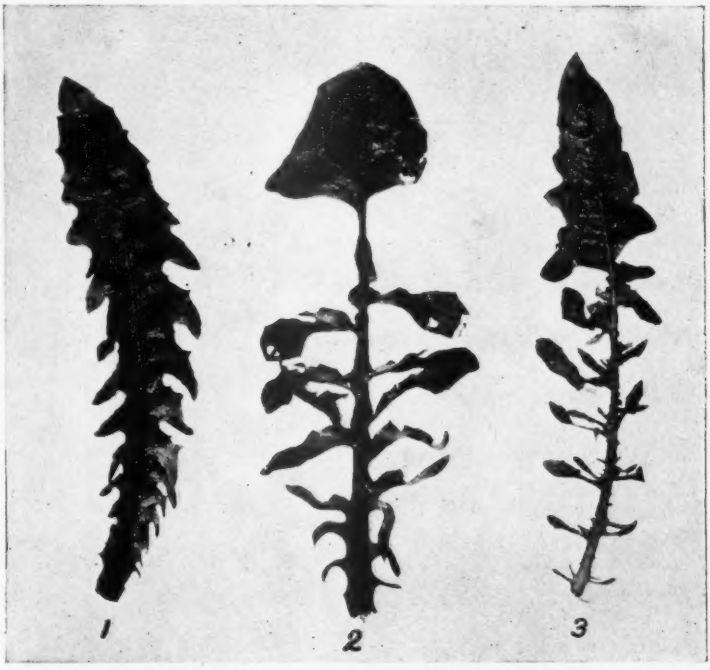

FIGURE 3.—Typical rosette leaves from 1, *C. biennis*, 2 *C. artificialis* and 3 *C. setosa*. Magnification approximately, ×1/2.

robust than the 24 chromosome plants of the same culture. The five achenes in the bottom group of figure 4 are from this triploid and are slightly larger than the average of the others which are from 24 chromosome plants.

It has been shown that *C. artificialis* contains 10 pairs of *biennis* and 2 pairs of *setosa* chromosomes. Meiosis in the triploid presented some

TABLE 2

A comparison of the principal characters of Crepis artificialis with those of C. setosa and C. biennis.

CHARACTER	*setosa* (n=4)	*biennis* (n=20)	*artificialis* (n=12)
Habit of growth	annual	biennial	annual
Stems	slender, bristly hairy, leafy, sharply angular, much branched.	stout, rough coarse hairy, and soft white tomentum, less leafy than *setosa;* branched in upper half.*	semi-stout, sparsely hairy angular, less leafy than *setosa*, branches on upper half.*
Height	23–60 cm.	45–120 cm.	30–60 cm.
Radical leaves	hairy, not rough, petiolate, often lyrate.	rough hairy, slightly petiolate only, seldom lyrate.	rough hairy, petiolate, lyrate.
Cauline leaves	sagittate and clasping	narrow only slightly clasping.	like *biennis*, narrow and clasping.
Peduncles	slender, angular, hispid with long hairs, and canescent puberulent.	stout, not hairy or only sparsely so.	stout, hispid with sparse long *setosa*-like hairs and wooly white tomentum.
Receptacle	alevolate, the alveolae small with short-ciliate margins, at maturity almost glabrous.	alveolate, alveolae large, with laciniately long fringed margins.	alveolate like *biennis* but less well developed

TABLE 2 cont'd

CHARACTER	*setosa*	*biennis*	*artificialis*
Heads	14–16 mm. long, 19 mm. in diameter when open. Involucral bracts hoary from a fine pubescence, all bracts setose hirsute with long yellowish glandless hairs, bracts keeled.	larger than *setosa*, 25–38 mm. diameter when open. Stiff short black hairs, sometimes glandular, on bracts Outer bracts have no keel, inner have weak keel about ½ its length.	25–38 mm. in diameter when open, pubescence of two kinds like *setosa* (not glandular), bracts keeled.
Florets	red sometimes appears on back of ligule, stamen-tube yellow, stigmas blackish green	no red on back of ligule, stamen tube yellow.	no red on back of ligule, stamen tube yellow stigmas dark greenish black.
Achenes	grayish brown, spindle shaped, beaked, 10 equal ribs, 2 sizes of achenes, 4 mm. and 5 mm.	dark or light brown, long columnar, slightly narrowed at tip, not beaked. 10–13 ribs with alternate stronger and weaker. Achenes average 5 mm. but show variation in size. Not two distinct size classes.	brown, spindle shaped, attenuate at both ends, sometimes a very short beak, 10–13 ribbed equal in size, size more like that of *setosa* with two means at 4 mm. and 5 mm.

* Old plants of these species also show branching from lower part of the plant.

unusual features. No trivalent chromosomes were found at diakinesis but cells showing 18, 19 and 20 chromatin units at the first metaphase were observed of which 19 was the most frequent number. These could clearly be distinguished as 17_{II} and 2_{I}. The plates with 18 units consisted of 17_{II} and 1_{I}, the other single probably being obscured. Where 20 units were observed there appeared to be four singles present. In some of the first metaphase plates singles appeared to be dividing. (This would give rise to the extra univalent chromosomes.) Here, as in the backcross of $F_1 \times$ *biennis*, the 30 *biennis* chromosomes form 15 pairs, while the 6 *setosa* chromosomes form 2 pairs and 2 singles instead of two trivalent units, although each *setosa* chromosome is certainly present three times.

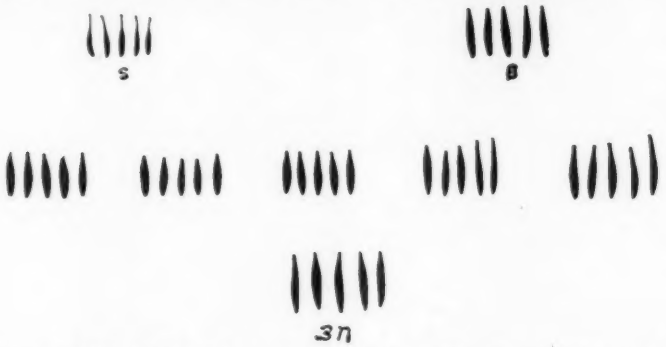

FIGURE 4.—Typical achenes of *C. setosa* (S), *C. biennis* (B), above, with achenes from six different plants of *C. artificialis*, the lowest group (3n) contains achenes from a triploid plant of *C. artificialis*. Magnification, ×1.5.

Triploids are usually very low in fertility but due to the large number of bivalent chromosomes (17) formed here, the fertility is quite high as indicated by the large amount of seed produced and from examination of anthers which showed only 30–38 percent bad pollen. The apparently good pollen showed variation in size.

The progeny of this triploid exhibited two distinct types in the seedling stage in about equal numbers. One group of 55 seedlings were normal in regard to cotyledon leaves; the other of 49 plants had abnormally developed cotyledon leaves, which were usually more or less coalesced. The two types are illustrated in figure 5. The rosettes of nearly mature plants exhibit much variation in size and type of leaves.

From the type of meiosis observed we should expect the progeny to have a range in chromosome number from 34 to 38. At the present time

the number in nine plants is as foliows: five with 34 chromosomes, two with 31 and one each with 33 and 35 chromosomes. The failure to find plants with 36 to 38 chromosomes was very probably correlated with the presence of micronulcei and microcytes frequently observed at the tetrad stage.

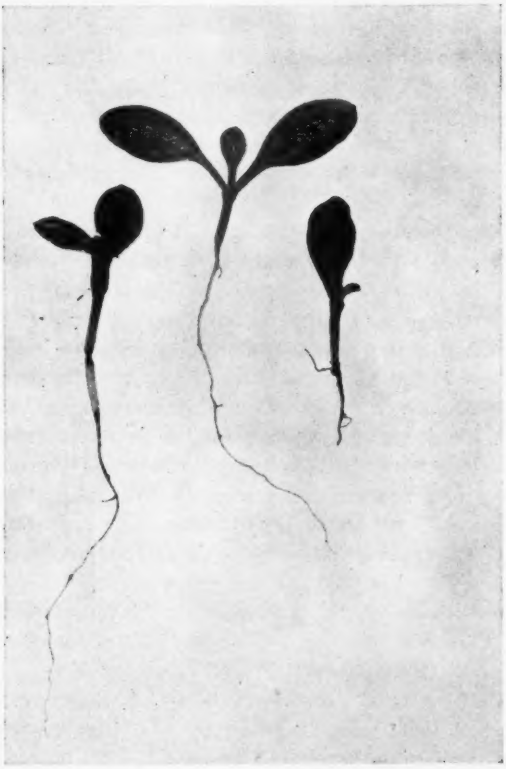

FIGURE 5.—Seedlings of the *C. artificialis* triploid plant showing a normal seedling and on each side seedlings with abnormal cotyledon development. Magnification, ×2.

We are not yet able to account definitely for the numbers lower than 34. The plants having 34 chromosomes should be the origin of a new form having 30 *biennis* chromosomes with 4 *setosa* chromosomes. They represent *artificialis* plants to which 10 additional *biennis* chromosomes have been added. Two of these 34 chromosome plants have been placed

in an isolated field at Davis, California, to determine their ability to reproduce under natural field conditions.

DISCUSSION

Crepis artificialis has the same number of chromosomes (24) as the *biennis*×*setosa* F_1 hybrid and each has 20 chromosomes from *biennis*. The F_1 has four univalent *setosa* chromosomes, while *artificialis* has two bivalent chromosomes from *setosa*. Although there is only this small difference in the chromosomes, the plants are quite distinct in phenotype. The F_1 plants are essentially *biennis* in appearance while *artificialis* shows some *setosa*-like characters more or less modified in their expression by the presence of some *biennis*-like characters. In the F_1 resulting from crossing *artificialis* with *setosa*, plants containing 10 chromosomes from *biennis* and 6 from *setosa* were secured. The latter included the two pairs present in *artificialis* plus one member of each of the two pairs not represented in *artificialis*. These plants were more like *setosa* than is *artificialis*.

Of the three outstanding distinctive characters of *setosa*, (a) pubescence (b) lyrate terminal lobe of the rosette leaves and (c) the beaked achenes, the first two are present in *artificialis* in a marked degree, while the third is not present or only to a very slight degree (see figure 4). In other hybrid strains from the same species cross beaked achenes are present, usually with no or only a small development of the *setosa* type of pubescence. Consequently it may be concluded that the major multiple genes for beak production are not present in *artificialis*, while those for pubescence must be largely located in the two pairs of *setosa* chromosomes included in the *artificialis* complex. (In the F_2 of *biennis*×*setosa* and subsequent generations there were plants showing different degrees of expression of beak and pubescence which suggested the operation of cumulative multiple genes for these characters).

These experiments have also added to our knowledge concerning the chromosomes of *biennis*. The normal diploid number appears to be 40 but plants of this species have been found to have 39, 41, 42, 43 and 45, (including counts made by M. NAVASHIN). LONGLEY (1927) has studied the occurrence of spernumerary chromosomes in maize where the normal diploid number is 20 but plants with one, two and three additional chromosomes have been found more or less frequently. These extra chromosomes appear to be repetitions of a single type, the smallest of the maize set. We have not been able to identify the extra chromosomes in *biennis* as repetitions of a single type but that chromosome elimination

occurs is evident from the occasional presence of microcytes in pollen formation in some strains of *biennis*.

This species has many of the characteristics of a polyploid. Its morphological characters can easily be considered as *gigas* forms of corresponding characters in some other species. M. NAVASHIN states that pentaploid forms of *capillaris* (n = 3) resemble *biennis* plants in some respects, (unpublished data). The mode of conjugation of the *biennis* chromosomes indicates that there are four homologous sets of chromosomes each consisting of five members. These sets probably differ to some extent in the genes they carry but retain sufficient homology to conjugate under appropriate conditions. This conjugation of 20 to form 10 pairs and of 10 to form 5 pairs leads to the conclusion that *biennis* is an octoploid form with 5 as the original haploid number of chromosomes. ROSENBERG (1920) found 42 chromosomes in the somatic cells and considered three as the basic number. He divided the chromosomes roughly into three size types, large, medium and small in approximately the numbers required by his hypothesis. The evidence from conjugation, however, does not lend support to this view. According to the octoploid hypothesis there should be four of each kind of chromosome, in the haploid *biennis* set. Some of the chromosomes exhibit marked individuality and appear to be present four times as expected, but it has not been possible to identify four of each of the recognizable types. A more detailed account of the chromosome types in *biennis* will be presented in a later paper.

In view of the polyploid nature of *biennis* it became of interest to examine the species of Crepis having five pairs of chromosomes to ascertain whether *biennis* gave evidence of being a polyploid derivative of an existing species. None of the eight species available, *Crepis aurea* L. Cass., *C. alpina* L., *C. foetida* L., *C. rubra* L., *C. sibirica* L., *C. leontodontoides* Alt., *C. tingitana* Salz., and *C. multicaulis* Ledeb., can at this time be recognized as the form from which *biennis* originated.

Crepis artificialis has characters sufficiently different from its parents and other species that it must be recognized as a distinct taxonomic unit. *It represents a connecting link between two widely separated species of the genus* and approaches the requirements of a good species as defined by VAN ESELTINE (1927), "Given a definite number of pairs of chromosomes and a perfectly regular method of cell division allied with distinct groups of other morphological characters, we have a good species."

New species which become established in nature must be hardy enough to mature and leave progeny. This new form of Crepis has been grown only under cultivation but appears equally as hardy as its parents. Ex-

periments are being conducted to determine its ability to survive under natural conditions.

There is abundant circumstantial evidence to indicate that new species are derived from intercrossing of existing species but the experimental verification of this is very meager and mostly the result of accident rather than design. The additional cases of experimentally produced new forms can be readily catalogued. In Viola, CLAUSEN (1926) obtained a fertile 14 paired species *V. hyperchromatica*, in the F_3 from the cross of *V. tricolor* (n = 13) with *V. arvensis* (n = 17). This form although constant for chromosome number exhibited considerable genic heterozygosity. Fertile constant forms have also appeared as the result of doubling of the chromosome complex of sterile F_1 species hybrids. CLAUSEN and GOODSPEED (1925) obtained a 72 chromosome form, *Nicotiana diguluta*, which contained a full diploid number from each of the two species, *N. tabacum* (n = 24) and *N. glutinosa* (n = 12). They suggested a similar origin of *Primula Kewensis* (n = 18) from *P. verticillata* (n = 9) and *P. floribunda* (n = 9). KARPECHENKO (1927) records the occurrence of a constant intergeneric hybrid having n = 18 chromosomes derived from crossing *Raphanus sativus* (n = 9) with *Brassica oleracea* (n = 9). A fertile Aegilops × wheat hybrid was reported by TSCHERMAK and BLEIER (1926) which had double the number of chromosomes found in the normally sterile F_1 hybrid. LJUNGDAHL (1924) obtained a constant F_1 hybrid from crossing *Papaver striatocarpum* (n = 35) with *P. nudicaule* (n = 7), the gametes of the F_1 all having 21 chromosomes. JORGENSEN (1928) obtained a tetraploid F_1 hybrid having a diploid number of *Solanum nigrum* and a diploid number of *Solanum luteum* chromosomes. Gamete formation exhibited occasional aberrations but was principally regular, although only 35 percent of good pollen was produced.

Many genera contain species which differ in the number and morphology of the chromosomes. In polyploid groups the increase appears to have resulted from the addition of haploid sets. In genera where no such polyploid series exist but the species differ by one or more pairs of chromosomes the methods resulting in such differences are obscure. Evidence from non-disjunction does not favor this as a method. However, in the results of crosses between species differing in number of chromosomes we have a method which can bring about just such results. The new Viola species, *V. hyperchromatica*, reported by CLAUSEN (1926), and the new species of Crepis reported here, represent two variations of this method of evolution.

It has been shown that species of Crepis will cross readily when grown in adjacent garden cultures, and indications of natural crossing between certain Western American species have been observed. There is ample justification for considering natural hybridization as a method of evolution in Crepis.

The formation of new species possessing chromosomal contributions from two or more species accounts not only for differences in number of chromosomes among species of a genus but also the differences in size and shape of the various chromosomes in a species.

It remains for such experimentally produced new forms to demonstrate their ability to survive under the conditions to which newly formed species in nature would be subjected. It is to be expected, however, that some would not be equal or superior to existing natural species. *Primula Kewensis* could undoubtedly maintain itself in the presence of the two parent species. In other cases, isolation from the parent forms would be necessary. This, however, does in no way discount the essential fact which has been demonstrated, namely, that new species differing in chromosome number and phenotypic characters result from species crossing.

An additional evident fact arising from a consideration of experimental results is that species are not necessarily the result of slow accumulations of minute mutations but may appear suddenly as a single individual in the F_1 or subsequent hybrid generations whenever the proper chromosome combination occurs.

SUMMARY

1. The *biennis* chromosomes in the F_1 produced by crossing with other species conjugate during gametogenesis to form 10 pairs. They are capable of further conjugation to form 5 pairs under proper conditions.

2. *Biennis* is a polyphoid species the haploid complex of which contains four sets of chromosomes each having five members.

3. The type of conjugation of the *biennis* chromosomes indicates an octoploid condition, yet each kind of chromosome is probably not present eight times.

4. *Crepis artificialis* is a new constant hybrid form containing 10 pairs of *biennis* and 2 pairs of *setosa* chromosomes.

5. The *setosa* chromosomes in *artificialis* can be identified as the long chromosome pair with a large satellite and the smallest pair of *setosa*.

6. *Artificialis* has characters which distinguish it from other species. It forms a connecting species (or link) between two subgeneric sections.

7. New species can rise from interspecific hybrids in various ways.

Such species appear suddenly and are not the result of a gradual change by successive accumulations of minute differences.

8. It has been shown that two pairs of chromosomes from one species can function regularly in a nucleus containing a portion of the chromosome number from a distinct species.

9. Trisomic sets of *setosa* chromosomes do not form trivalent groups at synapsis but instead bivalent and univalent units are formed.

This study has been supported by grants from the Board of Research of the UNIVERSITY OF CALIFORNIA.

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